EVALUATION OF PREY COMPOSITION AND NUTRITIONAL VALUE OF DIETS OF FREE-RANGING HARBOR SEALS (*PHOCA VITULINA*) FROM TUGIDAK ISLAND, ALASKA

By

Gretchen L. Geiger

RECOMMENDED:

Dr. James Carpenter

Dr. Larissa Horstmann-Dehn

Ms. Kate Wynne

Dr. Shannon Atkinson
Advisory Committee Chair

Dr. Katrin Iken
Head, Program in Marine Science and Limnology

APPROVED:

Dr. Michael Castellini
Dean, School of Fisheries and Ocean Science

Dr. John Eichelberger
Dean of the Graduate School

11/29/2012
Date

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A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

By

Gretchen L. Geiger, B.S.

Fairbanks, Alaska

December 2012

ABSTRACT

Changes in climate can cause shifts in ecosystem structure that can affect quantity or quality of prey available to predator populations. Due to sex or age-specific behaviors of predators, certain classes within a population may be more severely impacted by changes in their diet. This study evaluated prey composition and nutritional value of summer diets of harbor seals (*Phoca vitulina richardii*) from Tugidak Island, Alaska from 2001-2009. The MIXIT-WIN program was used to estimate the nutritional value of average harbor seal diets. Changes in relative abundance of certain prey species were correlated to sea surface temperature anomalies. Despite changes in prey composition, the nutritional value of the average harbor seal diet did not change. Fecal corticosteroid metabolite profiles were analyzed to identify age and sex of individual harbor seals from scats. Profiles obtained from a known adult male harbor seal could be differentiated from those of known adult female and juvenile male seals. Similar profiles were observed in unknown age and sex samples. Even though diet diversity differed between these groups, the nutritional quality of consumed diets was not significantly different. Tugidak Island harbor seals have flexible diets allowing them to capitalize on available prey to maintain their nutritional intake.

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ACKNOWLEDGEMENTS

First and foremost I would like to thank Dr. Shannon Atkinson for all of her guidance and support and for providing me with the opportunity to embark on this Alaskan-sized, graduate school adventure. I would also like to extend a huge thank you to Angie Steeves for helping me out with anything and everything I could possibly need. From help with paperwork, to finding housing, to simply chatting through tough points, I would not have made it this far without either of you. Thank you to all of my committee members, Dr. James Carpenter, Dr. Lara Horstmann-Dehn, and Kate Wynne for all of your direction and dedication to assisting me through this degree.

To Kendall Mashburn, thank you so much for your patience and all-around laboratory prowess, which helped me persevere and carry on through all those tedious days (and nights) spent in the lab. Special thanks also to Dr. Jason Waite for both his statistical expertise (without which I would still be writing R scripts) and his friendship, for which I am truly grateful. I would like to thank Gail Blundell, Kelly Hastings and Lauri Jemison from the Alaska Department of Fish and Game (ADF&G) for insightful discussion on harbor seals at Tugidak Island and Sue Goodglick, Tom Gage, and Carol Wilson (also of ADF&G) for collecting scats on Tugidak Island. I also thank Justin Smith and Jon Barton (of ADF&G) for assistance with sample cleaning. For the donation of known sex and age harbor seal scat samples, thank you to the staff (and harbor seals) of the Alaska Zoo, especially Shannon Jenson. Also thank you again to Dr. James Carpenter for donation of additional known sex and age harbor seal scat samples.

To all of the friends that I have made here in Juneau and throughout Alaska over the course of this adventure, thank you all so much for being my "family away from home" and for all of the support and also the fun that we have had throughout these past few years. Last, but certainly not least, I would like to give a huge hug and thank you to my family for giving me a

shoulder to cry on, an ear to vent to, a laugh when I needed it, and for relighting the fire and the stubbornness in me to see this all through. I would never have even made it onto the plane up here without you! Love you guys!

This study was funded by the Pollock Conservation Cooperative Research Center (to SA and GLG), Alaska Department of Fish and Game (to GMB), and National Marine Fisheries Service (to KW).

GENERAL INTRODUCTION

There are five recognized subspecies of harbor seals ranging throughout much of the northern hemisphere. *Phoca vitulina richardii* are found from the eastern Aleutian Islands through the southern coast of Alaska, and along the Pacific coast of North America down to Baja, California (Reeves et al. 2002, Burns 2009). Harbor seals forage and mate in the water but come ashore at various haul-out sites to pup and molt in two distinct periods during the summer, which lasts from May to September. Prior to the 1970s, Tugidak Island (56°27'04"N, 154°01'05"W) in the western Gulf of Alaska (Figure 1.1) was the location of haul-out sites that had among the largest concentrations of harbor seals in the world (Pitcher 1990). Nearly 17,000 seals were counted in the area in the mid-1950s, but the total population was likely larger (Mathisen & Lopp 1963). Based on biological rates (e.g., fecundity rates and mortality rates) of the population and harvest records, it is estimated that up to 20,500 animals may have utilized these beaches as haul-out sites for pupping and molting during the mid-1960s (Pitcher 1990).

Between 1976 and the late 1980s the harbor seal population at Tugidak Island declined sharply (Pitcher 1990, Jemison et al. 2006). It is estimated that the population declined by as much as 85% between 1976 and 1988, with a maximum yearly population reduction of 19% from 1976 to 1982 (Pitcher 1990). As of the mid-1990s, the population stabilized and began to exhibit a moderate recovery (Jemison & Pendleton 2001, Small et al. 2003, Jemison et al. 2006). The number of seals hauling out on Tugidak increased at approximately 8.3% per year and 3.4% per year between 1994 and 2000 for the pupping and molting periods, respectively (Jemison et al. 2006) and 6.6% per year from 1993 to 2001 for the entire population surrounding Kodiak Island, including Tugidak Island (Small et al. 2003). Though the population continues to increase, the harbor seal population at Tugidak Island is still reduced from its historic numbers (Jemison et al. 2006).

The reasons behind this dramatic population decline have yet to be fully elucidated. One prevailing theory as to the cause of the decline and slow recovery of certain pinniped populations is the 'nutritional stress hypothesis.' This hypothesis states that a change in the basic components of pinniped diets was caused by either a reduction in prey availability or prey quality and subsequently contributed to declines (Alverson 1991). Nutritional stress is defined as an adverse physiological or behavioral state that results from sub-optimal foraging opportunities (Trites & Donnelly 2003). Nutritional stress may manifest itself in the form of reduced body size, condition, and natality rates, increased pup and juvenile mortality, decreased immune response and subsequent increased susceptibility to disease, and modifications to behavior (Rosen & Trites 2000, Atkinson et al. 2008), all of which can contribute to population declines.

There are multiple avenues by which a change in diet quantity or quality for pinnipeds can occur. Two prominent theories for driving changes to the prey base are, 1) competition of pinnipeds with fisheries that may lead to localized depletion of prey stocks, and 2) climate oscillations that may lead to changes in prey distribution. Competition with fisheries for prey resources can have direct impacts on harbor seals. If harbor seals and commercial fisheries are primarily targeting the same species then there is increased potential for the target species to become depleted (Jemison & Kelly 2001). Likewise, changes in prey distribution and community composition resulting from climate oscillations (Anderson & Piatt 1999) can impact the normal foraging habitat of seals and other predators relying on these resources. They will then have to turn to other prey, which may be less beneficial for the predator. Therefore, it is important to understand what prey items are found in pinniped diets. Even though available prey biomass may be high, if these prey are not of sufficient nutritional quality, pinnipeds may become subject to nutritional stress.

Harbor seals are generalist predators and throughout their range they consume a wide variety of prey from both pelagic and benthic sources. This includes small, schooling, forage fishes, larger, piscivorous fishes, cephalopods, and crustaceans (Pitcher 1980, Tollit et al. 1998, Jemison 2001, Berg et al. 2002, London et al. 2002, Andersen et al. 2004, Lance & Jeffries 2007, Wright et al. 2007). While many different prey species may be found in the diets of harbor seals, often only a few of these species will be consistently and frequently identified in diet analyses and are assumed to dominate the typical diet (Pitcher 1980, Jemison 2001, Andersen et al. 2004). These dominant prey species can vary regionally, seasonally, annually, and also individually based on differential needs, preferences, or abilities of seals of differing age or sex classes (Pitcher 1980, Thompson et al. 1989, Coltman et al. 1997, Thompson et al. 1998, Le Boeuf et al. 2000, Jemison 2001, Andersen et al. 2004, Page et al. 2005, Beck et al. 2007). Ultimately, changes in the prey base leading to a change in availability or quality of important prey species may influence the success of seal populations.

Average harbor seal diet composition, as defined by prey species composition and the nutritional composition, may also change within a sampled year depending on the time of year the sample was collected. Harbor seals haul out on land during two specific time periods (i.e., in the early and late summer) to pup and to molt. At Tugidak Island, the breeding season is from May to the beginning of July when pups are weaned, while the molting period extends from the end of July through September (Jemison & Kelly 2001, Daniel et al. 2003). During these times, seals are hauled out more frequently and for longer periods than during other times of year and may employ central-place foraging strategies to maintain a tie to their haul-out (Burns 2009). Harbor seals exhibit sex-specific behavioral patterns, likely resulting from different reproductive demands, that could impact their foraging strategy and therefore prey access (Thompson et al. 1989, Boness et al. 1994, Thompson et al. 1994, Boness & Bowen 1996, Coltman et al. 1997,

Boness et al. 2006). It is therefore critical to evaluate sex-specific differences in prey resource exploitation when assessing potential population effects of prey changes.

Even if prey is available in abundance, the quality, nutritional composition, and digestibility of these prey items can have impacts on pinnipeds. Quality of harbor seal diet can be described as its nutritional value, as estimated by the combined nutritional value of prey species consumed. Seasonal, regional, and even individual variation in prey nutritional composition can further complicate nutritional studies. For example, both herring (*Clupea pallasi*) and walleye pollock (*Theragra chalcogramma*) exhibit seasonal changes in energy density and herring can be less energy dense than walleye pollock (3.48 kJ g^{-1} vs. 5.41 kJ g^{-1}, respectively), depending on when and where these fish are sampled (Kitts et al. 2004, Vollenweider et al. 2011).

The MIXIT-WIN computer software program (Agricultural Software Consultants Inc., San Diego, California) can be used to evaluate the quality of diets of harbor seals with regard to protein content, lipid content, ash content, and gross energy (GE). By comparing the quantitative plane of nutrition in observed diets, it is possible to assess how changing prey composition may affect overall nutritional quality of diets.

The prey remains found in fecal samples ("scats") can be used to investigate potential changes in diet composition of Tugidak Island harbor seals. Scat analysis is a common, cost-effective, non-invasive method to evaluate the diet composition of captive and free-ranging pinnipeds (Pitcher 1980, Cottrell et al. 1996, Merrick et al. 1997, Berg et al. 2002, Andersen et al. 2004, Casper et al. 2006, Tollit et al. 2006). The frequency of prey species' occurrence in scats is assumed to portray the relative frequencies that these prey items will have in a sampled diet (Berg et al. 2002, Andersen et al. 2004). Many studies have quantified the biases associated with the complete or partial digestion of prey hard-parts (da Silva & Neilson 1985, Jobling & Breiby 1986, Harvey 1989, Tollit et al. 1997, Bowen 2000, Orr & Harvey 2001, Arim & Naya 2003,

Tollit et al. 2003, Trites et al. 2005, Tollit et al. 2006, Phillips & Harvey 2009). For example, differential fragility and digestion of prey hard parts may lead to under-representation or absence of some prey species in the diet analysis (da Silva & Neilson 1985, Tollit et al. 1997, Bowen 2000, Orr & Harvey 2001, Berg et al. 2002, Tollit et al. 2003, Andersen et al. 2004, Phillips & Harvey 2009). Size of prey species may also be underestimated due to the dissolution of bones during digestion. Use of a variety of bones and other hard structures (i.e., not just otoliths), some of which are less susceptible to digestion, can reduce the degree to which species with more fragile remains are overlooked (Cottrell et al. 1996, Tollit et al. 2003, Tollit et al. 2006, Phillips & Harvey 2009). Cephalopod beaks may become concentrated in pinniped stomachs (Pitcher 1980) and may consequently be regurgitated when they are unable to be passed, leading to their underrepresentation in scat samples (Pitcher 1980, Trites et al. 2007, Sigler et al. 2009) . Due to differential retention times and passage rates of certain prey species within a predator, prey items from a single meal may be defecated at different times and parts of a single prey item may be defecated over the course of several scats (Prime 1979, Harvey 1989, Bowen 2000, Orr & Harvey 2001, Tollit et al. 2006, Phillips & Harvey 2009). This may result in a single scat having a disproportionate amount of prey hard-parts, skewing interpretation of the importance of certain prey species. With the additional assumption that each scat is representative of one meal or foraging bout, all diet assessments of prey occurrence should be viewed as minimum estimates.

When collected over time and in multiple areas, scat analysis can be used to depict diet over temporal and spatial scales (Tollit et al. 2003, Andersen et al. 2004). Although harbor seals utilize Tugidak Island as a haul-out site year round, the greatest numbers are present during their summer pupping and molting periods (Jemison & Kelly 2001, Daniel et al. 2003). Therefore, the most convenient opportunity to collect a large number of scats coincides with these two events,

both of which occur during the summer months from May to September (Jemison & Kelly 2001, Daniel et al. 2003).

Tagging studies of pups from Tugidak Island demonstrated that pups travel away from the Tugidak haul-out sites where they were first tagged (Small et al. 2005). During such extended foraging trips, harbor seals may defecate in the water or on other haul-outs precluding collection of scat samples. Thus, prey species that are processed faster than others could be missed in analysis of later scats. The average passage rate for harbor seals (the length of time when a majority of the meal should have passed through the seal's digestive system) is approximately 24 hours (Stanberry 2003, Phillips & Harvey 2009). Thus, if harbor seals partake in extended foraging trips in which they travel longer than 24 hours to return to haul-out sites, prey composition of the average diet may be mis-represented as prey consumed nearer to shore are more likely to be deposited in scats on haul-outs.

The overall goal for this study was to describe the diet of harbor seals from Tugidak Island in terms of prey composition and nutritional value, and to explore a variety of factors that could cause diet to change over time. These factors include temperature oscillation in the Gulf of Alaska, population structure (i.e., sex or age group), and season when diet was examined (i.e., breeding or molting period). I hypothesized that harbor seal prey composition and nutritional quality will change and that these changes can be related to climatic shifts among the years (Chapter 1), season of diet examination within a year (Chapter 1), and sex or age class of the animal (Chapter 2). Furthermore, I predicted that the method used to estimate the relative importance of prey species will impact the interpretation of diet composition and subsequently, nutritional value.

LITERATURE CITED

Alverson DL (1991) Commercial fisheries and the Steller sea lion (*Eumetopias jubatus*): The conflict arena. Fisheries Research Institute, School of Fisheries, University of Washington, Seattle, WA, p99

Andersen SM, Lydersen C, Grahl-Nielsen O, Kovacs KM (2004) Autumn diet of harbour seals (*Phoca vitulina*) at Prins Karls Forland, Svalbard, assessed via scat and fatty-acid analyses. Canadian Journal of Zoology 82:1230-1245

Anderson P, Piatt J (1999) Community reorganization in the Gulf of Alaska following ocean climate regime shift. Marine Ecology Progress Series 189:117-123

Arim M, Naya DE (2003) Pinniped diets inferred from scats: analysis of biases in prey occurrence. Canadian Journal of Zoology 81:67-73

Atkinson S, Demaster DP, Calkins DG (2008) Anthropogenic causes of the western Steller sea lion *Eumetopias jubatus* population decline and their threat to recovery. Mammal Review 38:1-18

Beck CA, Iverson SJ, Bowen WD, Blanchard W (2007) Sex differences in grey seal diet reflect seasonal variation in foraging behaviour and reproductive expenditure: evidence from quantitative fatty acid signature analysis. Journal of Animal Ecology 76:490-502

Berg I, Haug T, Nilssen KT (2002) Harbour seal (*Phoca vitulina*) diet in Vesterålen, North Norway. Sarsia - North Atlantic Marine Science 87:451-461

Boness D, Bowen W, Oftedall O (1994) Evidence of a maternal foraging cycle resembling that of otariid seals in a small phocid, the harbor seal. Behavioral Ecology and Sociobiology 34:95-104

Boness DJ, Bowen WD (1996) The evolution of maternal care in pinnipeds. Bioscience 46:645-654

Boness DJ, Bowen WD, Buhleier BM, Marshall GJ (2006) Mating tactics and mating system of an aquatic-mating pinniped: the harbor seal, *Phoca vitulina*. Behavioral Ecology and Sociobiology 61:119-130

Bowen W (2000) Reconstruction of pinniped diets: accounting for complete digestion of otoliths and cephalopod beaks. Canadian Journal of Fisheries and Aquatic Sciences 57:898-905

Burns JJ (2009) Harbor seal and spotted seal. In: Perrin WF, Würsig B, Thewissen JGM (eds) Encyclopedia of Marine Mammals. Academic Press, San Francisco

Casper R, Gales N, Hindell M, Robinson S (2006) Diet estimation based on an integrated mixed prey feeding experiment using *Arctocephalus* seals. Journal of Experimental Marine Biology and Ecology 328:228-239

Coltman DW, Bowen W, Boness DJ, Iverson SJ (1997) Balancing foraging and reproduction in the male harbour seal, an aquatically mating pinniped. Animal Behaviour 54:663-678

Cottrell P, Trites A, Miller E (1996) Assessing the use of hard parts in faeces to identify harbour seal prey: results of captive-feeding trials. Canadian Journal of Zoology 74:875-880

da Silva J, Neilson JD (1985) Limitations of using otoliths recovered in scats to estimate prey consumption in seals. Canadian Journal of Fisheries and Aquatic Sciences 42:1439-1442

Daniel RG, Jemison LA, Pendleton GW, Crowley SM (2003) Molting phenology of harbor seals on Tugidak Island, Alaska. Marine Mammal Science 19:128-140

Harvey J (1989) Assessment of errors associated with harbour seal (*Phoca vitulina*) faecal sampling. Journal of Zoology 219:101-111

Jemison L (2001) Summary of harbor seal diet data collected in Alaska from 1990-1999. Alaska Department of Fish and Game, Division of Wildlife Conservation, Anchorage, AK, p314-322

Jemison LA, Kelly BP (2001) Pupping phenology and demography of harbor seals (*Phoca vitulina richardii*) on Tugidak Island, Alaska. Marine Mammal Science 17:585-600

Jemison LA, Pendleton GW (2001) Harbor seal population trends and factors influencing counts on Tugidak Island, Alaska. Alaska Department of Fish and Game, Division of Wildlife Conservation, Anchorage, AK, p31-52

Jemison L, Pendleton G, Wilson C, Small R (2006) Long-term trends in harbor seal numbers at Tugidak Island and Nanvak Bay, Alaska. Marine Mammal Science 22:339-360

Jobling M, Breiby A (1986) The use and abuse of fish otoliths in studies of feeding habits of marine piscivores. Sarsia 71:265-274

Kitts D, Huynh M, Hu C, Trites A (2004) Season variation in nutrient composition of Alaskan walleye pollock. Canadian Journal of Zoology 82:1408-1415

Lance MM, Jeffries SJ (2007) Temporal and spatial variability of harbor seal diet in the San Juan Island archipelago. SeaDoc Society, Washington Department of Fish and Wildlife, Lakewood, WA, p29

Le Boeuf B, Crocker D, Costa D, Blackwell S, Webb P, Houser D (2000) Foraging ecology of northern elephant seals. Ecological Monographs 70:353-382

London JM, Lance MM, Jeffries SJ (2002) Observations of harbor seal predation on Hood Canal salmonids from 1998 to 2000. NOAA Grant No. NA17FX1603, WDFW, PSMFC, p20

Mathisen OA, Lopp RJ (1963) Photographic census of the Steller sea lion herds in Alaska, 1956-1958. United States Fish and Wildlife Service, Special Scientific Report of Fisheries 424

Merrick RL, Chumbley MK, Byrd GV (1997) Diet diversity of Steller sea lions (*Eumetopias jubatus*) and their population decline in Alaska: a potential relationship. Canadian Journal of Fisheries and Aquatic Sciences 54:1342-1348

Orr AJ, Harvey JT (2001) Quantifying errors associated with using fecal samples to determine the diet of the California sea lion (*Zalophus californianus*). Canadian Journal of Zoology 79:1080-1087

Page B, McKenzie J, Goldsworthy SD (2005) Dietary resource partitioning among sympatric New Zealand and Australian fur seals. Marine Ecology Progress Series 293:283-302

Phillips EM, Harvey JT (2009) A captive feeding study with the Pacific harbor seal (*Phoca vitulina richardii*): Implications for scat analysis. Marine Mammal Science 25:373-391

Pitcher K (1980) Stomach contents and feces as indicators of harbor seal, *Phoca vitulina*, foods in the Gulf of Alaska. Fishery Bulletin 78:797-798

Pitcher KW (1990) Major decline in number of harbor seals, *Phoca vitulina richardsi*, on Tugidak Island, Gulf of Alaska. Marine Mammal Science 6:121-134

Prime J (1979) Observations on the digestion of some gadoid fish otoliths by a young common seal. International Council for the Exploration of the Seas Conference and Meeting Documents:1-3

Reeves RR, Stewart BS, Clapham PJ, Powell JA (2002) Guide to marine mammals of the world. AA Knopf Inc., New York, p118-121

Rosen D, Trites A (2000) Pollock and the decline of Steller sea lions: testing the junk-food hypothesis. Canadian Journal of Zoology 78:1243-1250

Sigler M, Tollit D, Vollenweider J, Thedinga J, Csepp D, Womble J, Wong M, Rehberg M, Trites A (2009) Steller sea lion foraging response to seasonal changes in prey availability. Marine Ecology Progress Series 388:243-261

Small R, Pendleton G, Pitcher K (2003) Trends in abundance of Alaska harbor seals, 1983-2001. Marine Mammal Science 19:344-362

Small RJ, Lowry LF, Hoef JM, Frost KJ, Delong RA, Rehberg MJ (2005) Differential movements by harbor seal pups in contrasting Alaska environments. Marine Mammal Science 21:671-694

Stanberry K (2003) The effect of changes in dietary fat level on body composition, blood metabolites and hormones, rate of passage, and nutrient assimilation efficiency in harbor seals. Master of Science, University of Hawaii at Manoa, Honolulu, HI, p116

Thompson P, Fedak M, McConnell B, Nicholas K (1989) Seasonal and sex-related variation in the activity patterns of common seals (*Phoca vitulina*). Journal of Applied Ecology 26:521-535

Thompson PM, Miller D, Cooper R, Hammond PS (1994) Changes in the distribution and activity of female harbour seals during the breeding season: implications for their lactation strategy and mating patterns. Journal of Animal Ecology 63:24-30

Thompson PM, Mackay A, Tollit DJ, Enderby S, Hammond PS (1998) The influence of body size and sex on the characteristics of harbour seal foraging trips. Canadian Journal of Zoology 76:1044-1053

Tollit D, Steward M, Thompson P, Pierce G, Santos M, Hughes S (1997) Species and size differences in the digestion of otoliths and beaks: implications for estimates of pinniped diet composition. Canadian Journal of Fisheries and Aquatic Sciences 54:105-119

Tollit D, Black A, Thompson P, Mackay A, Corpe H, Wilson B, Parijs S, Grellier K, Parlane S (1998) Variations in harbour seal *Phoca vitulina* diet and dive-depths in relation to foraging habitat. Journal of Zoology 244:209-222

Tollit D, Wong M, Winship A, Rosen D, Trites A (2003) Quantifying errors associated with using prey skeletal structures from fecal samples to determine the diet of Steller's sea lion (*Eumetopias jubatus*). Marine Mammal Science 19:724-744

Tollit D, Heaslip S, Deagle B, Iverson S, Joy R, Rosen D, Trites A (2006) Estimating diet composition in sea lions: which technique to choose? In: Trites A, Atkinson S, DeMaster D, Fritz L, Gelatt T, Rea L, Wynne K (eds) Sea lions of the world. Alaska Sea Grant College Program, University of Alaska, Fairbanks, AK, p293–308

Trites AW, Donnelly CP (2003) The decline of Steller sea lions *Eumetopias jubatus* in Alaska: a review of the nutritional stress hypothesis. Mammal Review 33:3-28

Trites AW, Joy R, Weckerly FW (2005) Dietary analysis from fecal samples: How many scats are enough? Journal of Mammalogy 86:704-712

Trites AW, Calkins DG, Winship AJ (2007) Diets of Steller sea lions (*Eumetopias jubatus*) in Southeast Alaska, 1933-1999. Fishery Bulletin 105:234-248

Vollenweider JJ, Heintz RA, Schaufler L, Bradshaw R (2011) Seasonal cycles in whole-body proximate composition and energy content of forage fish vary with water depth. Marine Biology:1-15

Wright BE, Riemer SD, Brown RF, Ougzin AM, Bucklin KA (2007) Assessment of harbor seal predation on adult salmonids in a Pacific Northwest estuary. Ecological Applications 17:338-351

CHAPTER 1: Evaluation of prey composition and nutritional value of diets of free-ranging harbor seals (*Phoca vitulina*) from Tugidak Island, Alaska in warm and cool climatic periods[1]

ABSTRACT

Oscillating cool and warm climatic periods can alter the basic structure of an ecosystem potentially impacting the dietary base of a predator. This can then have detrimental effects on predator populations. The objectives for this study were to evaluate how harbor seal diet composition changes based on a variety of factors including collection season, climatic period, and diet estimation method. Harbor seal scats were collected from Tugidak Island, Alaska, during summers from 2001-2009. Prey remains recovered from scats were identified to the lowest possible taxonomic level and approximate prey composition of the diet was determined from split-sample frequency of occurrence and biomass calculations. The nutritional profile of estimated diets was determined using a prey nutritional database developed from proximate analyses of various prey found in Alaskan waters. The diet showed some changing prey proportions across collection years, but only the consumption of gunnels and *Gymnocanthus* spp. was significantly correlated with sea surface temperature anomalies. However, none of these prey species contributed largely to harbor seal diet over the collection period (each present in less than 10% of scats). None of the nutritional parameters (crude protein, lipid, ash, and gross energy) of the average harbor seal diet were correlated with climatic period or exhibited any trend associated with the breeding and molting periods. Combining the prey database and nutritional composition of diets across years indicated that harbor seals are generalist predators with flexible diets that can take advantage of abundant prey to maintain nutritional input despite climate oscillations.

[1] Geiger, G.L., S. Atkinson, J.N. Waite, G.M. Blundell, J.R. Carpenter, and K. Wynne. 2012. Prey and nutritional composition of harbor seal diets in warm and cool climatic periods in Alaska. Prepared for submission in Marine Ecology Progress Series.

INTRODUCTION

Historically, Tugidak Island, AK had one of the largest concentrations of harbor seals in the world with up to 17,000 seals present in the mid-1950s (Mathisen & Lopp 1963, Pitcher 1990). However, between the mid-1970s and the early 1980s, pinniped numbers throughout Alaska, including those on Tugidak Island, declined substantially (Pitcher 1990, Jemison et al. 2006). It is estimated that the Tugidak Island harbor seal population declined by as much as 85% between 1976 and 1988, with a maximum yearly population reduction of 19% per year from 1976 to 1982 (Pitcher 1990). During the 1990s their numbers stabilized and began to exhibit a recovery of about 6.6% per year from 1993 to 2001 (Small et al. 2003). Though this trend continues, the population is still reduced far below its pre-1970s size (Jemison et al. 2006). The reasons for this dramatic decline are not fully understood. A dominant theory in other Alaskan pinnipeds is that a dietary shift from high-quality prey to nutritionally inferior prey led to nutritional stress that contributed to population declines (Alverson 1991, Rosen & Trites 2000, Trites & Donnelly 2003).

Climatic regime shifts can alter the basic structure of the ecosystem, including prey species available to harbor seals, and therefore, may also have led to the population decline of harbor seals. In the late 1970s, a substantial change in prey species composition of the North Pacific was noted (Anderson & Piatt 1999, Mantua & Hare 2002). Food web structure of the North Pacific shifted from a web dominated by a variety of forage fishes and shrimps to one dominated by large, piscivorous fishes such as walleye pollock (*Theragra chalcogramma*) (Anderson & Piatt 1999). While large-scale climatic oscillations affect both the distribution and recruitment of prey (Beamish & Bouillon 1993, McGowan et al. 1998, Mantua & Hare 2002), shorter-term shifts in regional climatic variables, such as sea surface temperature (SST), also can

influence prey species (Mueter et al. 2002). Study of these smaller, more frequent shifts allows researchers to view the potential impact of future climate change on predator-prey relationships.

Within a given year, diets may vary as a consequence of harbor seal life history as well as prey seasonality. Harbor seals are opportunistic predators, readily capitalizing on a wide variety of prey species from both benthic and pelagic habitats, including sculpins, flatfishes, cephalopods, salmon (*Oncorhynchus* spp.), sand lance (*Ammodytes hexapterus*), and walleye pollock (*Theragra chalcogramma*) (Pitcher 1980, Tollit et al. 1998, Jemison 2001, Berg et al. 2002, London et al. 2002, Andersen et al. 2004, Lance & Jeffries 2007, Wright et al. 2007). However, while many prey species have been identified in diets, often only a few of these prey items are present consistently enough to be considered principal prey items in the typical harbor seal diet (Pitcher 1980, Jemison 2001, Andersen et al. 2004). Prior studies of Tugidak Island harbor seals have identified walleye pollock, Irish lord (*Hemilepidotus* spp.), and sand lance as dominating prey items (Pitcher 1980, Jemison 2001). However, these studies collected samples throughout the year and neither study examined potential seasonal differences in diet. Harbor seals employ central-place foraging strategies around haul-out locations during early and late summer when they spend time ashore while pupping and molting (Thompson et al. 1989, Burns 2009). Prey available to seals may be restricted by the seal's diminished ability to take extended foraging trips to foraging sites due to this seasonal tie to haul-out sites (Thompson et al. 1994, Coltman et al. 1997, Boness et al. 2006, Burns 2009). As a result, this may increase the potential for localized prey depletion as intraspecific competition for nearby resources increases, as has been observed with Pacific walruses (*Odobenus rosmarus divergens*) (Ray et al. 2006).

Fecal (scat) analysis is a common, cost-effective, and relatively non-intrusive method for diet analysis (Pitcher 1980, Cottrell et al. 1996, Merrick et al. 1997, Berg et al. 2002, Andersen et al. 2004, Casper et al. 2006, Tollit et al. 2006). Diet is estimated by evaluating the relative

frequencies of prey species identified in scats, which is assumed to represent the relative importance of these prey in the average harbor seal diet (Berg et al. 2002, Andersen et al. 2004). Biases associated with dietary scat analysis include underestimation of prey importance due to partial or complete digestive dissolution of prey with fragile or non-existent hard parts; multiple studies have been conducted to quantify these biases (da Silva & Neilson 1985, Harvey 1989, Tollit et al. 1997, Bowen 2000, Arim & Naya 2003, Trites et al. 2005, Tollit et al. 2006, Phillips & Harvey 2009). Use of scats to analyze diets is further complicated by differential passage rates of prey species through the harbor seal digestive tract. Some prey species (e.g., walleye pollock), may be passed at a slower rate than others across a larger number of scats (Harvey 1989, Cottrell et al. 1996, Bowen 2000, Tollit et al. 2003). Prolonged retention time in the digestive tract also increases the potential for these hard parts to become digested past identification (Harvey 1989, Cottrell et al. 1996, Bowen 2000, Tollit et al. 2003). In addition, some prey hard parts, such as cephalopod beaks, may be preferentially regurgitated leading to their underrepresentation in scat samples (Pitcher 1980, Trites et al. 2007, Sigler et al. 2009). Differential rates of passage can also lead to overestimation of relative prey importance, with prey items from a single meal defecated at different rates over several scats (Prime 1979, Harvey 1989, Bowen 2000, Orr & Harvey 2001, Tollit et al. 2006). Thus, collection of a single scat sample from an individual seal may not contain ratios of prey hard parts that are representative of a single consumed meal but represents a snapshot of the individual's diet at a single point in time. Scat analysis does not describe a complete diet profile. But these data provide an index of average harbor seal diet when combined with other scats from the same species, region, and time period.

Methods used to assess the relative importance of prey in the diet may also skew the outcome. Split-sample frequency of occurrence (ssFO) and biomass reconstruction (BR) are two commonly used dietary calculation methods for pinnipeds (Antonelis et al. 1997, Merrick et al.

1997, Tollit et al. 1998, Andersen et al. 2004, Page et al. 2005, Middlemas et al. 2006, Dehn et al. 2007, Lance & Jeffries 2007, Trites et al. 2007, Sigler et al. 2009, Waite et al. 2012), each with its own set of associated biases and assumptions. A major source of bias in use of ssFO calculations is that this method only considers presence and absence of prey and functions under the assumption that 1) identified prey species represent all prey items consumed in the last foraging bout, and 2) all of these prey species were consumed in equal quantities (Olesiuk et al. 1990). Biases associated with ssFO calculations include a tendency to overestimate the importance of smaller prey items compared to larger prey items (e.g., one sand lance may not be the dietary equivalent of one salmon). Similarly, prey items with robust hard-parts that are defecated over several scats may have higher prevalence in collected scats (e.g., walleye pollock) (Arim & Naya 2003, Tollit et al. 2003). On the other hand, estimates using BR account for the differential quantities and mass of prey consumed. Both ssFO and BR have potential for the underestimation (or complete oversight) of prey with more fragile or no hard parts (da Silva & Neilson 1985, Harvey 1989, Tollit et al. 1997, Orr & Harvey 2001, Tollit et al. 2006). However, with BR there is the additional concern for the overestimation of the relative importance of large prey items due to the assumption that the entire prey body was consumed when in reality only a portion was eaten (Laake et al. 2002, Wright et al. 2007, Hauser et al. 2008, Phillips & Harvey 2009). Use of BR estimations further assumes that length-weight equations for prey species are known (often not the case for less studied prey or understudied regions) and do not change over time or space.

The objectives of this study were to 1) describe the summer diet of harbor seals from Tugidak Island from scats from 2001 to 2009, 2) assess potential differences in diets of Tugidak Island harbor seals during the breeding and molting periods in 2008, 3) compare the relative importance of prey species in diets calculated using two different methods (i.e., ssFO and BR), 4) calculate the nutritional profile of harbor seal diets, and 5) determine if changes in prey

composition or nutritional profile correlate with oscillations in SST anomalies surrounding Tugidak Island and throughout the Gulf of Alaska.

METHODS

Sample Collection and Processing

Harbor seal scats were collected by the Alaska Department of Fish and Game (ADF&G) from the western beaches of Tugidak Island, Alaska (Figure 1.1) during summer (May to September) 2001-2009 (Table 1.1). Samples collected between May 1 and July 14 were considered part of the breeding season (Jemison & Kelly 2001), while scats collected between July 15 and September 30 were assigned to the molting period (Daniel et al. 2003). Breeding period samples were collected only during even-numbered years, while molting period scats were collected each year, 2001-2009. Scat samples for this study were collected under NMFS permit numbers 358-2585 and 358-1787.

Fresh seal scats were collected in ziplock bags and frozen at -20°C until further processing. Frozen scats were thawed, washed through a set of nested sieves (1000 μm, 710 μm, 500 μm) using commercially available dish-soap to break up the scat without damaging the prey remains. Suspected prey hard-parts were picked out of the sieve mesh using forceps and all hard-parts collected from each scat sample were placed in individual ziplock bags.

These isolated prey remains were then sent to Pacific IDentifications Inc. (PacID; British Columbia, Canada) to be identified to the lowest possible taxonomic level using an extensive reference collection. For all samples, PacID provided species identifications, including confidence codes for positive identifications, listing of specific structures used to make the identification, structure condition, the minimum number of individuals (MNI) of each prey species per scat, and estimates of the approximate length of each individual identified based on a

prey reference collection. Scats not containing identifiable prey items (i.e., unidentified fish or "empty" samples) were removed and not considered for further analyses.

Cephalopod beaks were individually examined and were all identified as giant Pacific octopus (*Enteroctopus dofleini*) using a combination of identification guides (Clarke 1962, 1986), photographs of beaks of giant Pacific octopus, and knowledge of the species of octopus commonly found around the Kodiak archipelago within diving depth limits of harbor seals (Conners & Conrath 2010). Hereafter, cephalopods will refer to giant Pacific octopus (GPO). Samples containing salmonids (*Oncorhynchus* spp.) were also re-examined following PacID analysis to increase taxonomic resolution using vertebrae width to height ratios (Huber et al. 2011). Chinook salmon (*O. tshawytscha*) was ascribed its own category, while pink (*O. gorbuscha*) and coho (*O. kisutch*) salmon were combined in one category, as were chum (*O. keta*) and sockeye (*O. nerka*) salmon (Huber et al. 2011). Because approximate salmon length was estimated based on unknown salmon species, all salmon biomass estimates consider them as "unidentified salmon". Species-specific data were applied at the nutritional level by taking the estimated total salmon contribution and calculating the approximate proportion of each salmon species' contribution to the average diet in a collection period based on vertebrate morphometric analysis.

Diet Assessment

Data returned from PacID were used to calculate the observed Shannon diversity index (SDI) for each collection period within each year. This is a commonly used index for evaluating diet diversity of pinnipeds (Sinclair & Zeppelin 2002, Trites et al. 2007, McKenzie & Wynne 2008, Herreman et al. 2009, Sigler et al. 2009, Waite et al. 2012). The SDI was calculated as follows:

$$H' = -\sum_{i=1}^{S} p_i \, (\ln p_i)$$

where H' is the SDI, p_i is the relative abundance of species i, and S is the number of species identified (Shannon 1949). A bootstrapping procedure (Crowley 1992) was used to estimate a mean SDI for breeding and molting period within each year and provide 95% confidence intervals so differences in diet diversity between breeding and molting periods could be evaluated.

The sFO and BR methods of estimating prey relative importance were only calculated for prey species that were identified in at least 10% of scats in at least one collection period. The ssFO was calculated for each prey species within a collection period as follows (Olesiuk et al. 1990):

$$\text{ssFO}_{jk} = \sum_{i=1}^{N} (\text{O}_{ki} / \sum_{k=1}^{\text{n}} \text{O}_{ki}) / N \quad k = 1, \ldots, \text{n (n = \# different prey species)}$$

where O_{ki} is a binary variable to indicate whether the kth species is present or absent (0 = absent, 1 = present) in the ith sample (Olesiuk et al. 1990). Therefore, if only a single species occurred in a sample it would be assigned a ssFO value of 1, whereas observation of two prey species in a sample, would score 0.5 for each, and so on. The sum of the scores for all species in all samples is represented by N (Olesiuk et al. 1990).

The ssFO for each species in each sample was then averaged across all samples within a collection period to represent the relative importance of a prey species in the average diet during that collection period. These values were interpreted as a species percent contribution to the average diet as the values summed to 1 (or 100%).

Estimates of biomass consumed by seals were calculated using length-weight regression equations for each species as established from the published literature (Appendix 1) and the

approximate length and minimum number of individuals (MNI) projected by PacID. Length-weight regression equations were not available for buffalo sculpin (*Enophrys* spp.), thus this species category was dropped from further consideration. To increase accuracy of BR calculations, individual bones or hard-parts from Irish lord (*Hemilepidotus* spp.; various bones) (Orchard 2003), GPO (beak morphometrics) (Robinson & Hartwick 1983), and Pacific halibut (*Hippoglossus stenolepis*; otoliths) (Southward & Hardman 1973) were measured using electronic digital calipers (Model: C20054: Range: 0-150 mm, Resolution: 0.01 mm, Accuracy: ±0.02 mm; Marathon: Richmond Hill, Ontario, Canada). Published equations were used to approximate the length (Irish lord and Pacific halibut) or weight (GPO) of the prey consumed. These species were selected because equations calculating approximate body length from bone measurements were available in the published literature (Southward & Hardman 1973, Robinson & Hartwick 1983, Orchard 2003) and were expected to provide a more accurate estimation of fish length than the broader size categories provided by PacID. These estimations were assumed to be more accurate because they were based on measurement of individual bones rather than the size of the skeletal reference collection at PacID. Accuracy for these species was especially important as these species could contribute a large portion to harbor seal diet (e.g., Irish lord), large variability in PacID size ranges was evident (e.g., small vs. very large halibut) and length/weight estimations were not provided in the data from PacID (i.e., GPO). For both ssFO and BR calculations, the average harbor seal diet for a given period was estimated to be the relative importance of all prey species in greater than 10% of scats identified in that collection period, averaged across all samples collected.

Nutritional Analysis

The relative importance of each prey species was calculated as percent contribution to the average diet for each collection period based on both sample ssFO and BR estimates, and

considering only prey species that occurred in ≥10% of scats in any collection period. Thus, by looking at percent contribution to average diet, the nutritional composition of the average diet as calculated by both ssFO and BR methods can be compared. Diet formulas were created to represent the average diet from each collection period for both ssFO and BR methods.

We used proximate composition data from the published literature for prey species found in Alaskan waters (Appendix 1) to evaluate the nutritional composition of the estimated diets. Whole-fish proximate composition data were imported into the MIXIT-WIN program (version 6.17, 2011; Agricultural Software Consultants Inc., San Diego, California). This program was originally designed to allow agricultural users to enter proposed diets to evaluate the nutritional quality and financial cost of these theoretical diets. The MIXIT program was used in this study to evaluate the quality and potential changes in quality of harbor seal diets with respect to crude protein, lipid, ash, and gross energy (GE) content. All nutritional parameters were evaluated on a wet-weight basis. This study describes the MIXIT program's first application for use with diets of free-ranging marine mammals. Proximate composition data were selected from studies with the largest sample sizes and sample collections closest to the Western Gulf of Alaska. When available, seasonal variation in prey nutrient composition data was taken into account (e.g., sand lance proximate composition in June compared to September; (Robards et al. 1999). Even though the gender and reproductive status of prey species can influence their nutrient composition, we were unable to determine these traits for prey items identified in scat samples. Thus, the nutrient composition of prey items in diet were all estimated on a mixed-sex basis.

Climate Data

Climate oscillations were observed through the use of sea surface temperature (SST) anomalies recorded in the Gulf of Alaska (GOA) waters where Tugidak Island harbor seals primarily reside and forage. SST anomalies were calculated from 2001 to 2009 using monthly

temporal composites from NOAA National Climatic Data Center's (NCDC) Extended Reconstructed Sea Surface Temperature (ERSST) data at two-degree spatial resolution (Smith et al. 2008). SST anomalies were calculated for waters surrounding Tugidak Island that were considered harbor seal foraging areas based on satellite tracking during the breeding and molting periods (Small et al. 2005). Sea surface temperature anomaly oscillations were also calculated for the entire GOA from 1997 to 2010[2] using Advanced Very High Resolution Radiometry (AVHRR) data to account for large-scale ecosystem processes throughout the GOA, which could have possibly impacted harbor seal prey survival and abundance outside of the Tugidak Island area. The SST data within each of these databases were averaged across the year ranges to set the "zero line" around which the yearly SSTs deviated to obtain the SST anomalies. The longer time series was used for the GOA AVHRR data to incorporate potential changes in temperature that might have affected fish species distribution and survival prior to this study.

Data Analysis

When making between-year comparisons, only samples collected during molting periods were used. Analysis of within-year diet comparisons, included only those scats collected in years when both breeding and molting periods were sampled.

A simple bootstrapping procedure (Crowley 1992) was employed to resample the data to make statistical comparisons between the SDI[3] for the breeding and molting periods. When

[2] Range of years only used to set what is considered the "zero" line for anomalies. Temperatures were averaged across this year range to set the zero line and then looked at SSTs within sample years to see where SST deviated from this zero line to get anomalies. Years before sample period were included to incorporate potential changes in temperature that might affect fish species distribution or survival before this study began.

[3] By using bootstrapping we create theoretical sample sets of the same number by re-sampling the data with replacement (i.e., the R script uses the observed samples and selects one for the "new" group of samples, resubmits it to the pool of potential samples and draws again, until the sample size is the same). This procedure of creating a new theoretical sample set is repeated 1000 times to get 1000 SDIs for theoretical populations. These theoretical populations are then used to get averages and standard deviations, and evaluate for statistical differences in the SDIs between groups.

comparing differences between breeding and molting seasons, only years with both breeding and molting data were used. The data were resampled with replacement 1000 times to estimate a mean SDI. 95% confidence intervals for mean pairwise differences[4] in the SDI (e.g., 2002 breeding samples were compared to 2002 molting samples, etc.) were calculated to assess differences in diet diversity between these two periods within a year.

When sample size allowed, Pearson's correlations were calculated among average percent contribution of each prey species to diet within a year (i.e., for the molting period), for both ssFO and BR calculations, and SST anomaly around Tugidak Island. Because certain prey species were not observed in the diets in every year, Pearson's correlations were not calculated for these species because their occurrence in diets was not consistent enough to calculate the correlation. Pearson's correlations were computed using molting period data only to determine if variation in prey species prevalence in harbor seal diets could be explained by changes in SST. Prey species that were moderately ($r^2 \geq 0.5$) and highly ($r^2 > 0.7$) correlated with SST were considered to be potentially biologically significant. A positive correlation between prey species and SST indicated that the relative importance of that prey species was found to increase during years that were classified as "warm years" while a negative correlation indicated that the relative importance of a prey species decreased during anomalously warm years. Pearson's correlation coefficients were also computed between SST anomaly and the nutritional parameters that were calculated by the MIXIT program for each of the estimated diets. Due to the small sample size of scats from 2001, these values were omitted when analyzing diet and SST correlations. Pairwise differences in nutritional parameters between breeding and molting seasons for both ssFO and BR estimation methods were compared using a Wilcoxon signed rank test (Wilcoxon 1945) due to

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[4] All years that contained both breeding and molting data were used in this analysis. However, because it is a pairwise analysis, 2002 breeding samples were compared to 2002 molting samples, 2004 breeding with 2004 molting, and so on, so that differences between years would not impact results.

sample size limitations. All statistical analyses were performed with R version 2.15.0 (The R Foundation for Statistical Computing, Vienna, Austria) with $p < 0.05$ considered significant unless otherwise noted.

RESULTS

Diet

A total of 708 scat samples were collected from Tugidak Island between 2001 and 2009 with a range of 17 (2001) to 146 (2002) scats collected within a single summer (Table 1.1). Harbor seals on Tugidak Island consumed a wide variety of prey species during summer with 60 different prey species identified across the entire collection period (Table 1.2). An average of 2.6 prey species were identified per scat (maximum 11 species per scat). Of these, 21 prey species or groups of prey species (e.g., all Irish lord species were pooled to one Irish lord category due to frequent inability to identify hard-parts at species-specific resolution) were found in at least 10% of scats during at least one sampling period over the collection period. Table 1.2 shows the mean, standard deviation, and range of each of these prey species' relative importance across all collection years.

The relative importance of prey species in diets varied depending on calculation method (i.e., ssFO and BR). When comparing prey species importance to diet based on ssFO vs. BR estimations, three general scenarios were observed for prey species identified throughout the collection period: Scenario 1 - ssFO was estimated as being of higher relative importance than BR calculations for approximately 57.1% of the species (Figure 1.2a); Scenario 2 – BR estimations estimated a higher relative importance of a prey species compared to ssFO for about 9.5% of prey species (Figure 1.2b); and Scenario 3 – neither ssFO nor BR estimations were consistently higher than the other for 33.3% of species (Figure 1.2c). The values graphed in

Figure 1.2 are the relative importance of a single prey species that were representative of each of the three different scenarios.

Climate Correlation

Prey species with at least a moderate correlation ($r^2 \geq 0.5$) between relative dietary importance and SST are shown in Figure 1.3. None of the prey species exhibiting a moderate positive correlation with SST anomalies were statistically significant using either ssFO or BR estimates (Figure 1.3a). Prey species showing moderate negative correlation with SST anomalies are illustrated in Figure 1.3b. Only gunnel species (using BR estimates only) and *Gymnocanthus* spp. (using ssFO estimates only) were highly correlated ($r^2 > 0.7$) and statistically significant ($p=0.04$ and $p=0.003$, respectively).

Species Diversity

Diet diversity of harbor seals on Tugidak Island was similar during summers from 2001-2009 (molting period range SDI=1.47-2.11; breeding period range SDI=1.67-1.87) (Figure 1.4). However, the observed SDI for breeding season collection periods was always lower than that year's corresponding molting period index (Figure 1.4a). Mean SDI, estimated using bootstrapping methods, also demonstrated lower diet diversity during the breeding season (Figure 1.4b). Bootstrapped 95% confidence intervals for mean pairwise differences in SDI between breeding and molting periods did not contain zero (-0.375, -0.025), therefore diet diversity during breeding and molting periods differed significantly ($p<0.05$).

Nutritional Composition of Diets

Nutritional composition of the average diet for a single summer varied by collection year, nutritional parameter examined, and method of diet estimation used (Figure 1.5). When diet was

calculated by ssFO methods, protein content of the average diet ranged from 14.27-16.08 %, lipid content ranged from 2.64-3.85 %, ash content ranged from 2.57-2.96 %, and GE density ranged from 4.23-5.15 kJ g^{-1} wet-weight. When BR estimations were used to calculate the nutrient content of the average diet protein content ranged from 13.47-17.49 %, lipid content ranged from 1.57-4.90 %, ash content ranged from 2.00-2.54 %, and GE density ranged from 2.74-5.74 kJ g^{-1}. Only the ash content of the average summer diet differed significantly when using ssFO and BR methods (p=0.004).

Correlations among the nutritional data and SST anomalies showed ash content (ssFO: 0.52, p=0.19) of prey to be moderately associated (r^2>0.5), although not statistically significant. All other nutritional parameters were not associated with SST. No consistent trend between the nutritional composition of breeding and molting season diets (e.g., GE not consistently higher in molting period compared to breeding period) was identified (Figure 1.6).

DISCUSSION

As generalist predators, harbor seals typically consume a wide variety of prey ranging from benthic to pelagic species including sculpins, flatfishes, salmon, sand lance, pollock, and various cephalopods (Pitcher 1980, Tollit et al. 1998, Jemison 2001, Berg et al. 2002, London et al. 2002, Andersen et al. 2004, Lance & Jeffries 2007, Wright et al. 2007). Frequently though, only a fraction of these prey species have been consistently identified in harbor seal diets and appeared to dominate the diet of the average harbor seal (Pitcher 1980, Jemison 2001, Andersen et al. 2004). For example, Pitcher (1980) observed 16 different prey categories in the diets of Tugidak Island harbor seals, but walleye pollock and capelin occurred in 35.9% and 11.4% of scats, respectively, while no other prey occurred in more than 10% of scats. These dominant prey items have varied by seal sex, by study area, and by collection season and year. Between 1975

and 1978, the diets of harbor seals on Tugidak Island were dominated by walleye pollock (Pitcher 1980), but between 1990 and 1999 the dominant prey items were found to be sculpins, specifically Irish lords, and sand lance (Jemison 2001).

Steller sea lions (SSL; *Eumetopias jubatus*) are also known generalist predators that frequently have dietary overlap with harbor seals (Pitcher 1981). Between 1999 and 2005, SSL around the Kodiak archipelago consumed diets predominantly consisting of sand lance, pollock, arrowtooth flounder, and Pacific cod (*Gadus macrocephalus*) (McKenzie & Wynne 2008). Due to the potential for dietary overlap between harbor seals and SSL, we expected to see similar prey species dominating harbor seal diets samples during our study period (McKenzie & Wynne 2008). In our study, prey dominating the average harbor seal diet during summers from 2001-2009 included Irish lord, greenling, rock sole (*Lepidopsetta* spp.), cephalopods (specifically GPO), salmon, and Pacific cod. In contrast to previous studies of harbor seals and SSL diets from the Kodiak archipelago (Pitcher 1980, McKenzie & Wynne 2008), walleye pollock did not contribute largely (<3%) to the average harbor seal diet . As harbor seals are opportunistic predators, less pollock in the overall diet may reflect a decrease in their local availability (Grigg et al. 2009). However, this could also reflect a possible preference for other prey and selection against pollock by harbor seals sampled in this study.

Within this study we observed that salmon comprised a large portion of the overall biomass consumed, but were consumed primarily during the late summer (between July and September). While salmon are present around Kodiak Island from May to October, availability of most salmon species increases as they return to natal streams to spawn in the late summer months (i.e., July-September) (Jackson & Dinnocenzo 2012). Salmon was not identified as a dominant prey item in previous studies of Kodiak area harbor seals, but this may be because of when samples were collected. In other studies, sample collections did not occur solely during the

summer months (i.e., during the time when salmon were plentiful or in the area) (Pitcher 1980, Jemison 2001). Therefore, sample collections that occurred during months when salmon were not readily available around Kodiak Island could lessen the estimated importance of salmon, especially when data from all seasons were evaluated together.

Similar to previous studies on harbor seals (Pitcher 1980, Jemison 2001, Andersen et al. 2004), 60 different prey species were identified in scats throughout the collection periods sampled in this study. Despite the large number of prey species, only a few were consumed in large quantities so the diet diversity, as reported by SDI for the collection period, remained low (below 2.15 for all years; highest possible SDI=20).

The relative importance of prey species that dominated the average harbor seal diet in a particular collection period changed among collection periods. However, only two prey species (i.e., gunnel and *Gymnocanthus* spp.) were significantly negatively correlated with SST. Changes in relative importance of prey possibly reflect changes in prey availability in response to changing climatic variables, such as SST. However, both species were only of minor importance to harbor seals around Tugidak Island (<10% of the diet) in this study and their fluctuations were therefore unlikely to cause any impact to the population.

The methodology used to analyze consumed diets can affect the interpretation of estimated relative importance of prey items. Three different categories were described to qualitatively represent the different patterns observed between estimates of relative importance of prey species by ssFO and BR calculations (Figure 1.2). While ssFO estimations were quicker and easier to calculate than BR estimations, they have been shown to overestimate the relative importance of smaller prey species compared to larger prey items, because the MNI and size of prey items are not accounted for (Olesiuk et al. 1990). Due to uncertainty and potential biases in consumed BR estimations, accurate estimations of consumed biomass were more challenging to

obtain. BR analysis, on the other hand, has potential to underestimate the biomass consumed because length-weight estimates may be calculated with partially or completely dissolved hard parts (da Silva & Neilson 1985, Harvey 1989, Tollit et al. 1997, Orr & Harvey 2001, Tollit et al. 2006). Further, lack of length-weight equations for many prey species often precluded the estimation of consumed biomass for all species identified in a sample, thus these species are underrepresented. Species for which equations were available may be assumed to be more important solely because their biomass could be estimated. Potential for overestimation of biomass also exists where only a portion of the prey item, especially if it is very large, was actually eaten (Laake et al. 2002, Wright et al. 2007, Hauser et al. 2008, Phillips & Harvey 2009). Despite these potential biases, BR estimation has been reported to provide a better reconstruction of the average diet of predators because it considers the amount of a prey species consumed and not merely presence or absence (Tollit et al. 2006, Phillips & Harvey 2009, Klare et al. 2011). Even though BR estimations were determined to be preferred, all analyses were conducted on both BR and ssFO estimations in order to identify areas where use of only one method would produce different results.

In the ssFO calculations we observed that Irish lord had a high relative importance for harbor seals throughout a majority of the time series, similar to Tugidak Island harbor seals between 1990 and 1999 (Jemison 2001). However, by solely relying on ssFO estimation for diet analysis, the importance of certain prey species that were consumed less frequently, but in high biomass (e.g., salmon) would have been overlooked. This may be one reason why salmon was considered to have high relative importance to overall diet in this study, but was not considered an important prey species in prior studies of Tugidak Island harbor seals (Pitcher 1980, Jemison 2001). This confirms that it is important to consider consumed BR estimations where possible

and more research is required to expand the library of available length-weight equations for different prey species and regions.

Several studies have been conducted to estimate the minimum number of scats required to obtain the most accurate diet estimations (Hammond & Rothery 1996, Trites et al. 2005). To accurately identify principle prey items occurring in >5% of scats, a minimum of 59 scats is recommended to be collected (Trites et al. 2005). This minimum number was achieved for approximately half of the collection periods (Table 1.1). Therefore, we expect that, for these collection periods, we were able to identify the principle prey items consumed by harbor seals during these periods.

While the reduction in diet diversity may be the result of small sample sizes collected during the breeding season, the behavior of harbor seals may also play a part. Harbor seals differ from other phocids in that females do not fast throughout the entire lactation period but instead, resume foraging partway through, more similar to otariids (Boness et al. 1994, Boness & Bowen 1996). This may be a consequence of the small body size of harbor seals, and them not being able to store sufficient energy reserves to sustain the high energy costs of lactation (Boness et al. 1994, Bowen et al. 2001). Females will thus resume foraging, while still caring for their pups. Even though pups will sometimes go on foraging trips with their mothers (Bowen et al. 1999), these trips are likely shorter and closer to haul-out sites than the trips the female will undertake at other times of the year when she does not have to care for a dependent pup (Thompson et al. 1994). Consequently, female harbor seals with pups may not be able to access diverse, preferred foraging locations, thereby potentially leading them to forage on a less diverse array of prey, closer to natal haul-out sites. Male harbor seals may also show a decrease in diet diversity during the breeding season. Harbor seals mate in the water and mating occurs following the birth of pups. During this period, when post-partum females resume foraging trips to supplement the costs

of lactation (Boness et al. 1994, Boness & Bowen 1996, Bowen et al. 2001), males may constrain their foraging trips in an attempt to maximize their mating opportunities (Coltman et al. 1997, Boness et al. 2006). Therefore, both sexes may exhibit a decrease in diet diversity during the early summer months coinciding with the breeding period, potentially as a consequence of breeding activities.

Despite changes to dominating prey species in diets across years and between the breeding and molting seasons, the nutritional value of the harbor seal diets did not change. Even though fluctuations occurred for the various nutritional parameters, the GE of the average diet from 2001 to 2009 did not vary. There was also no discernible trend in the fluctuation of the nutritional parameters between the breeding and molting periods, despite changes to prey composition and subsequent decrease in diet diversity as determined by SDI. Therefore, even though the average prey composition of diets changed throughout the time series, harbor seals appeared to be capable of maintaining their energetic intake during these shifts in SST and prey availability.

The maintenance of energy intake, in spite of fluctuating species composition of diet, may be a factor of the mixed diet that the harbor seals consume. By not specializing in the capture of one particular prey item, harbor seals are able to readily take advantage of whatever prey they are able to locate and capture. Captive feeding studies have demonstrated that harbor seals exhibit diet flexibility allowing them to compensate for lower quality prey items by increasing gut fill, increasing digestive efficiency, and augmenting protein and lipid assimilation into their tissues (Stanberry 2003, Trumble et al. 2003, Trumble & Castellini 2005, Zhao et al. 2006). Therefore, even in times where preferable prey are unavailable, harbor seals may be able to maintain an energetic balance.

The energy density of a prey species can vary substantially between seasons (Robards et al. 1999, Kitts et al. 2004, Vollenweider et al. 2011). For instance, even though walleye pollock and herring are generally considered to have low and high energy densities, respectively, this is not the case throughout the entire year. Both walleye pollock and herring energy density have been observed to change seasonally, and low energy herring (3.48 kJ g^{-1}) was found to be less energy dense than high energy pollock (5.41 kJ g^{-1}) (Kitts et al. 2004, Vollenweider et al. 2011). Therefore, before ascribing a "blanket assignment" of low or high energy density to a particular prey species, the seasonal, regional, and even individual, variation in energy densities of different prey need to be carefully evaluated.

While this study demonstrates the MIXIT program's potential usefulness in dietary studies of pinnipeds, there are several challenges with the data gained from this program. For many fish species proximate composition data are limited. The lipid content and consequently the GE density of many fish species can vary depending on when and where samples were collected (e.g., sand lance energy density in spring and early summer is about 25% greater than in late summer and fall due to roe production in females) (Robards et al. 1999). However, for most prey species, these data are not available. Because the MIXIT program is only as useful as the data entered into the database, more proximate composition data for various prey species during different times of year and different regions are required to expand the nutritional database used for these types of studies. There is also potential for prey proximate composition to change annually, so continued analysis of the nutritional parameters of prey species is required to evaluate how the nutritional quality of harbor seal diets changes with the changing prey base.

Nutritional estimations may be skewed by preferential consumption of specific prey parts. Certain prey species, especially large prey items such as halibut and salmon, may not be entirely consumed (Wright et al. 2007, Hauser et al. 2008, Phillips & Harvey 2009). Such partial

prey consumption can lead to overestimation of consumed biomass and can affect interpretation of nutritional data. In the present study, we assumed that all of a given prey item was consumed. However, harbor seals are known to preferentially consume the energy rich bellies and roe of female salmon and subsequently discard the remainder of the carcass (Hauser et al. 2008), a practice that has also been observed to occur in bears (*Ursus arctos* and *U. americanus*) (Gende et al. 2001). Harbor seals from Iliamna Lake, Alaska were observed to consume nearly the entire body of male salmon in 96.6% of cases, but usually leaving the head (Hauser et al. 2008). In contrast, when female salmon were consumed, the whole body, excluding the head, was eaten in only 31.3% of cases (Hauser et al. 2008). Instead, seals seemed to prefer to consume the bellies of female salmon in 63.6% of cases, which were assumed to contain energy-rich eggs due to their proximity to spawning locations (Hauser et al. 2008). Thus, there is not only potential for overestimation of consumed biomass and skewing of estimated energy density of consumed prey (Gende et al. 2004, Hauser et al. 2008), but if harbor seals only target the soft bellies and eggs of female salmon, no hard parts may be consumed and these predation events may be completely undetectable in scat analysis. Combining scat analysis with other methods of diet estimation, such as stomach content, fatty acid, or stable isotope analysis may provide a more complete diet profile. Direct field observations of harbor seal feeding, especially the handling of large prey items such as salmon, may also allow quantification of the potential bias associated with actually consumed vs. discarded prey.

Harbor seals are generalist predators with flexible diets and can readily take advantage of available prey to maintain their nutritional input. Future studies should attempt to expand the proximate composition database for prey in different times of the year, in order to refine analysis of the nutritional quality of diets. This study provides baseline data for the diets of harbor seals during the summer months, for a population of harbor seals that is currently increasing. Should

the Tugidak Island harbor seals experience another decline, future diet studies can use the data presented herein to evaluate changes in prey composition or nutritional quality of diets that might contribute to the decline and either help support or dismiss the nutritional stress hypothesis.

FIGURES

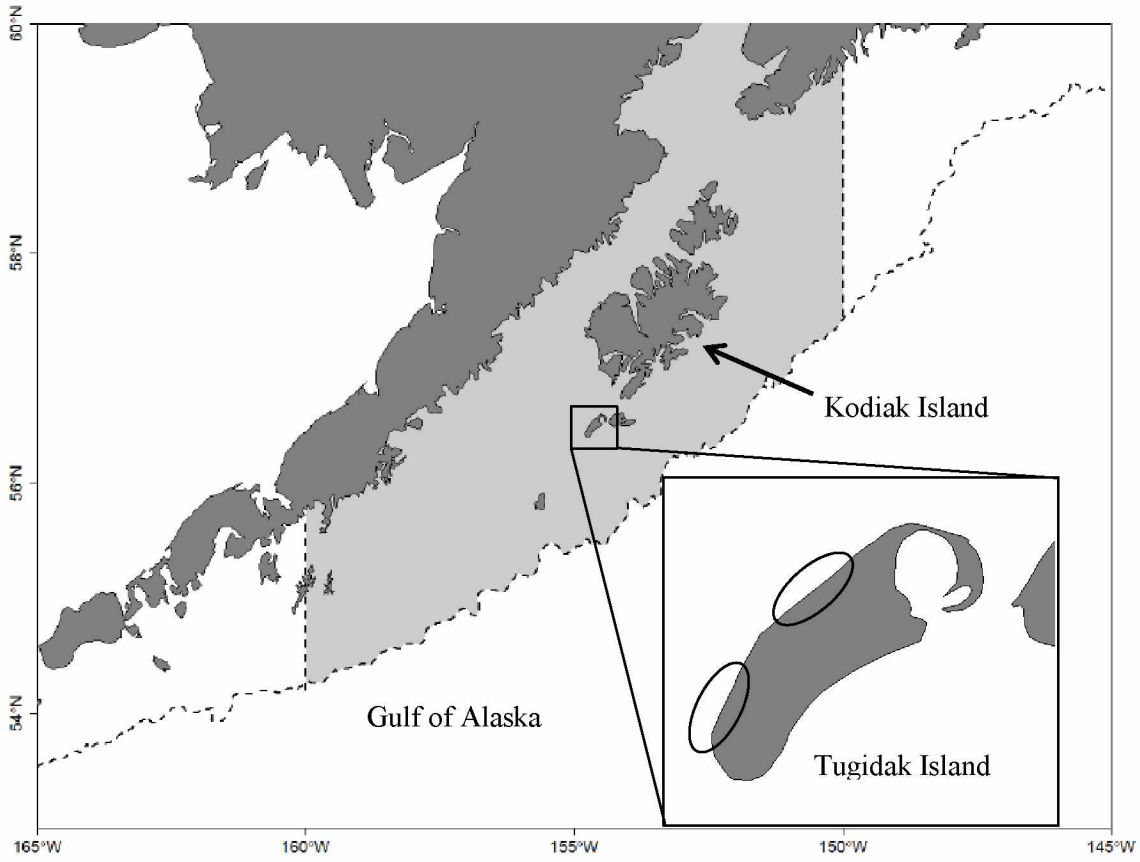

Figure 1.1: Location of Tugidak Island in the western Gulf of Alaska. The area for which sea surface temperature (SST) anomalies were calculated from is shown in the light grey shaded area shown on the larger map. The SST anomaly data were collected from Extended Reconstructed Sea Surface Temperature (ERSST) data and were determined to be waters that these seals were immediately moving to and foraging in. Scat samples were collected from the middle and southwest beaches shown by the ovals in the inset.

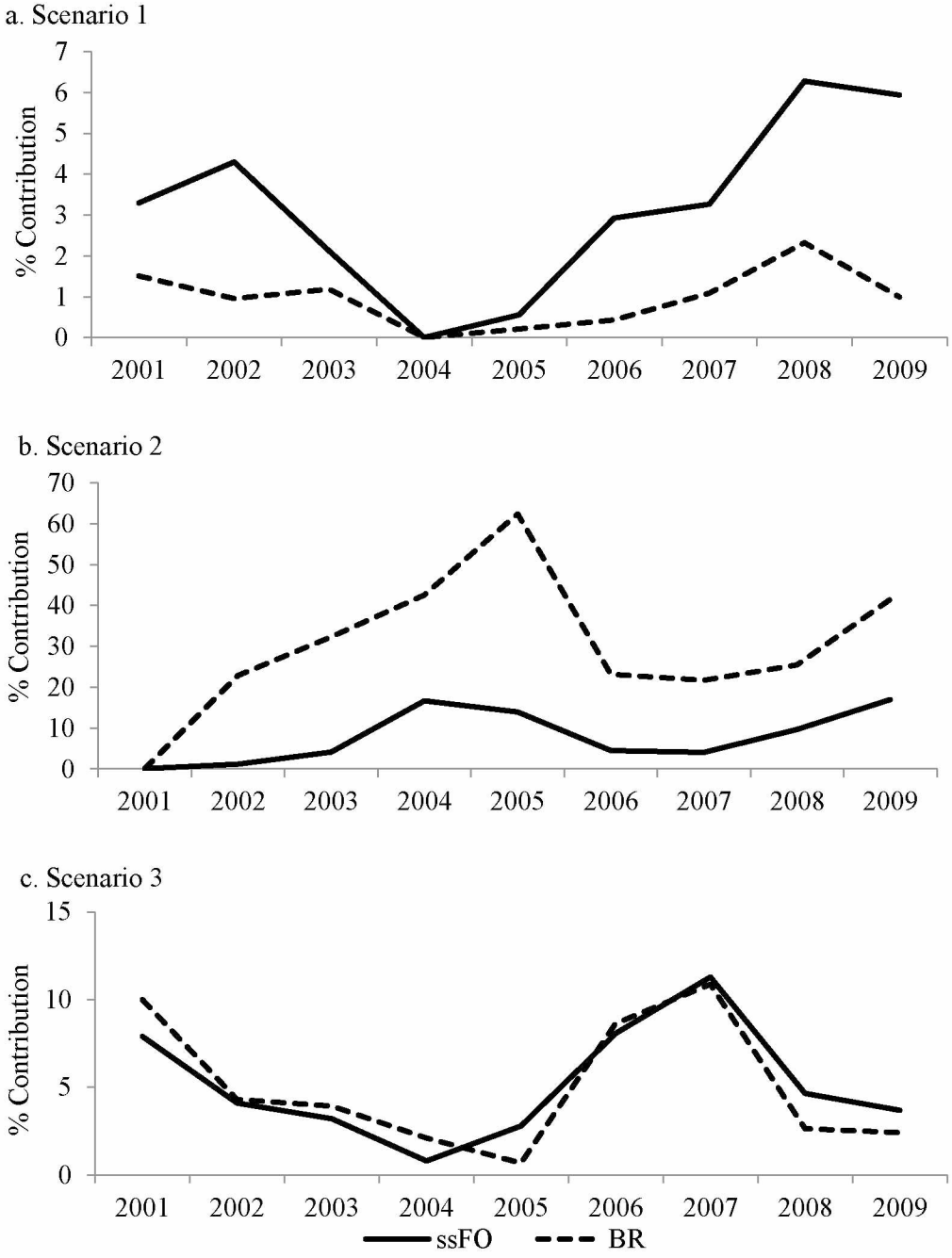

Figure 1.2: Variation in the differences between estimation of prey relative importance based on method of calculation. a) Scenario 1: split-sample frequency of occurrence (ssFO) calculations estimated higher prey species contribution to average diet than biomass (BR) calculations (57.1% of species), b) Scenario 2: BR calculations resulting in higher estimation of prey species relative importance in average diet than compared to ssFO (9.5% of species), and c) Scenario 3: neither ssFO nor BR calculations produced consistently dominating estimations of prey species (33.3% of species).

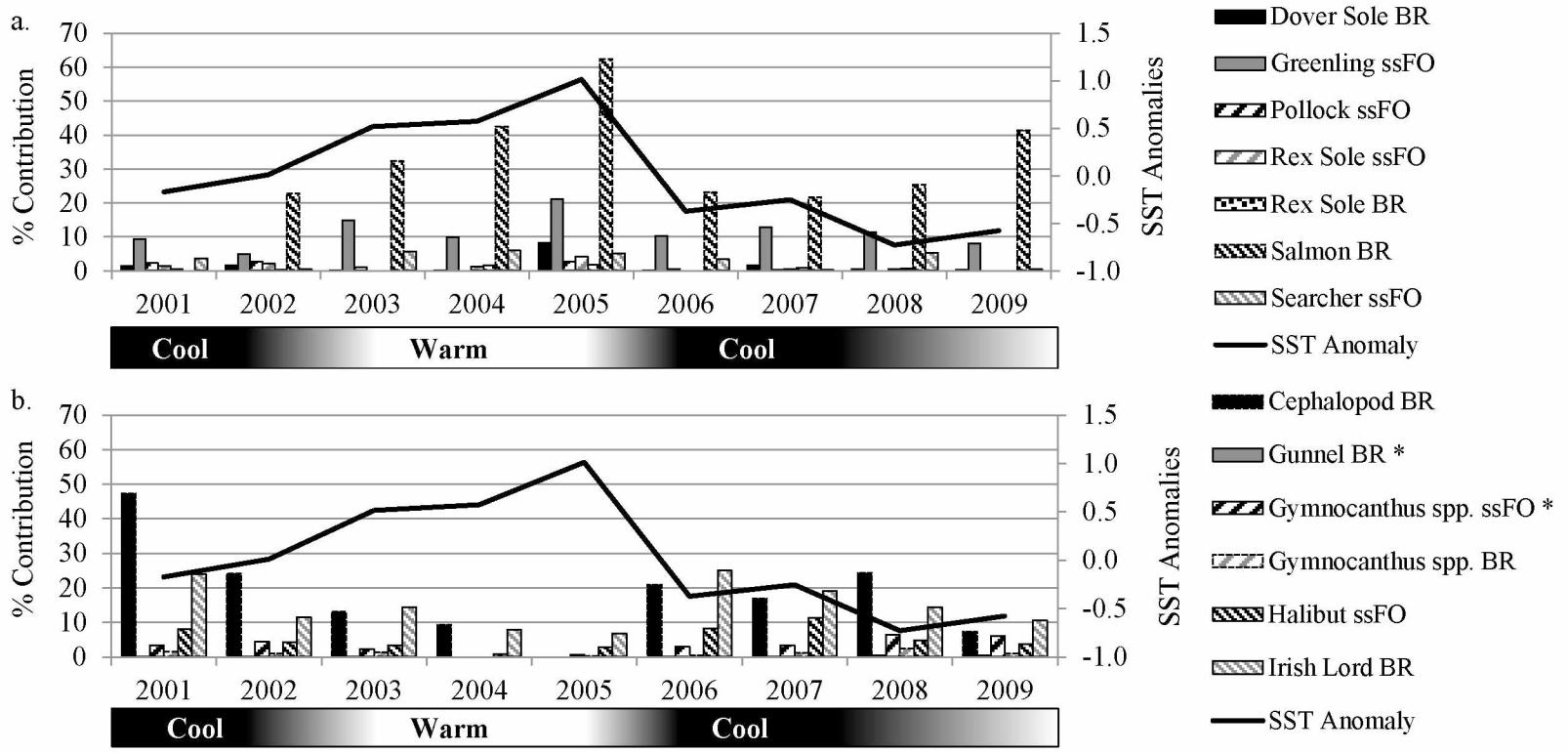

Figure 1.3: Pearson's correlation between prey species relative importance and climate. Prey species relative importance in harbor seal (*Phoca vitulina*) molting season diet from 2002 to 2009 correlated with sea surface temperature (SST) anomalies calculated in the Gulf of Alaska (GOA) (moderate correlation $r^2 \geq 0.5$; *, $p < 0.05$). 2001 data were excluded from correlation analysis due to small sample size. Prey species relative importance was calculated either by split-sample frequency of occurrence (ssFO) or biomass reconstruction (BR) methods. a) Prey species with increasing contribution to harbor seal diets during "warm" climatic periods, and b) prey species with increasing contribution to harbor seal diets during "cool" climatic periods. SST anomaly line was calculated from Extended Reconstructed Sea Surface Temperature (ERSST) data from 2001-2009 in the waters surrounding Tugidak Island. The gradient bar underneath each graph represents the overall climatic period observed throughout the entire GOA from 1997-2010 using Advanced Very High Resolution Radiometry data.

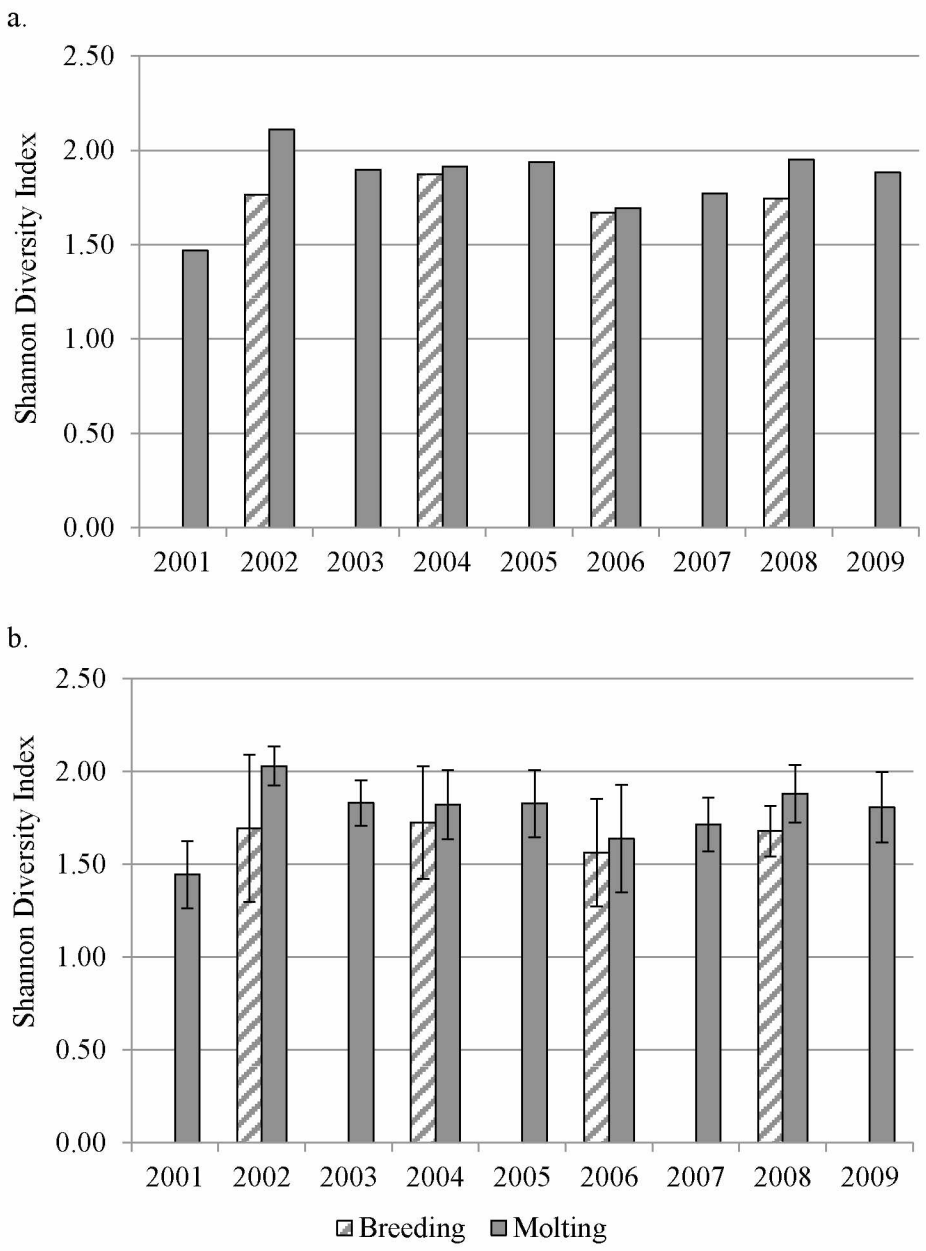

Figure 1.4: Shannon diversity index calculated for harbor seal (*Phoca vitulina*) scats. Scats were collected during the breeding (May - early July) and molting (late July - September) periods throughout the collection time series (2001 - 2009). a) Observed Shannon diversity index for the breeding and molting periods. b) Mean Shannon diversity index estimated by bootstrapping. Error bars represent 95% confidence intervals as estimated by bootstrapping. Bootstrapped 95% confidence intervals for mean pairwise differences between the breeding and molting seasons do not contain zero ($p < 0.05$), therefore breeding season diet diversity was observed to be significantly lower than molting season diversity.

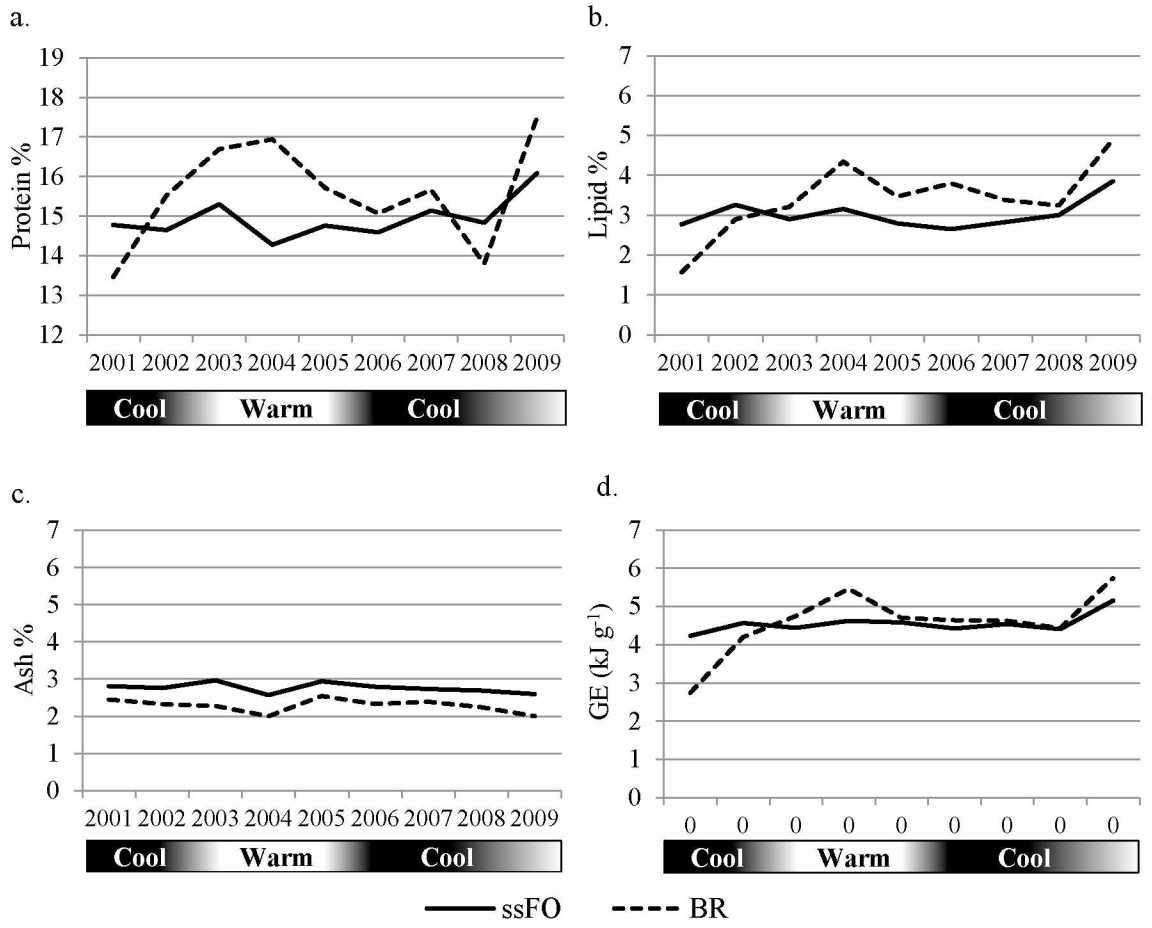

Figure 1.5: Predicted nutrient composition of estimated diets of harbor seals (*Phoca vitulina*). Nutritional data predicted using scats collected during the molting periods throughout the 2001-2009 sampling period calculated using split-sample frequency of occurrence (ssFO) and biomass reconstruction (BR) calculations. Proximate composition of estimated diets were estimated by the MIXIT-WIN program (Agricultural Software Consultants Inc., San Diego, California); a) protein content (%), b) lipid content (%), c) ash content (%), and d) gross energy (GE) content (kJ g^{-1}). All nutritional parameters were estimated on a wet-weight basis. Climatic period, as defined by sea surface temperature (SST) anomalies calculated for the Gulf of Alaska from 1997-2010, is depicted in the gradient bar beneath each graph.

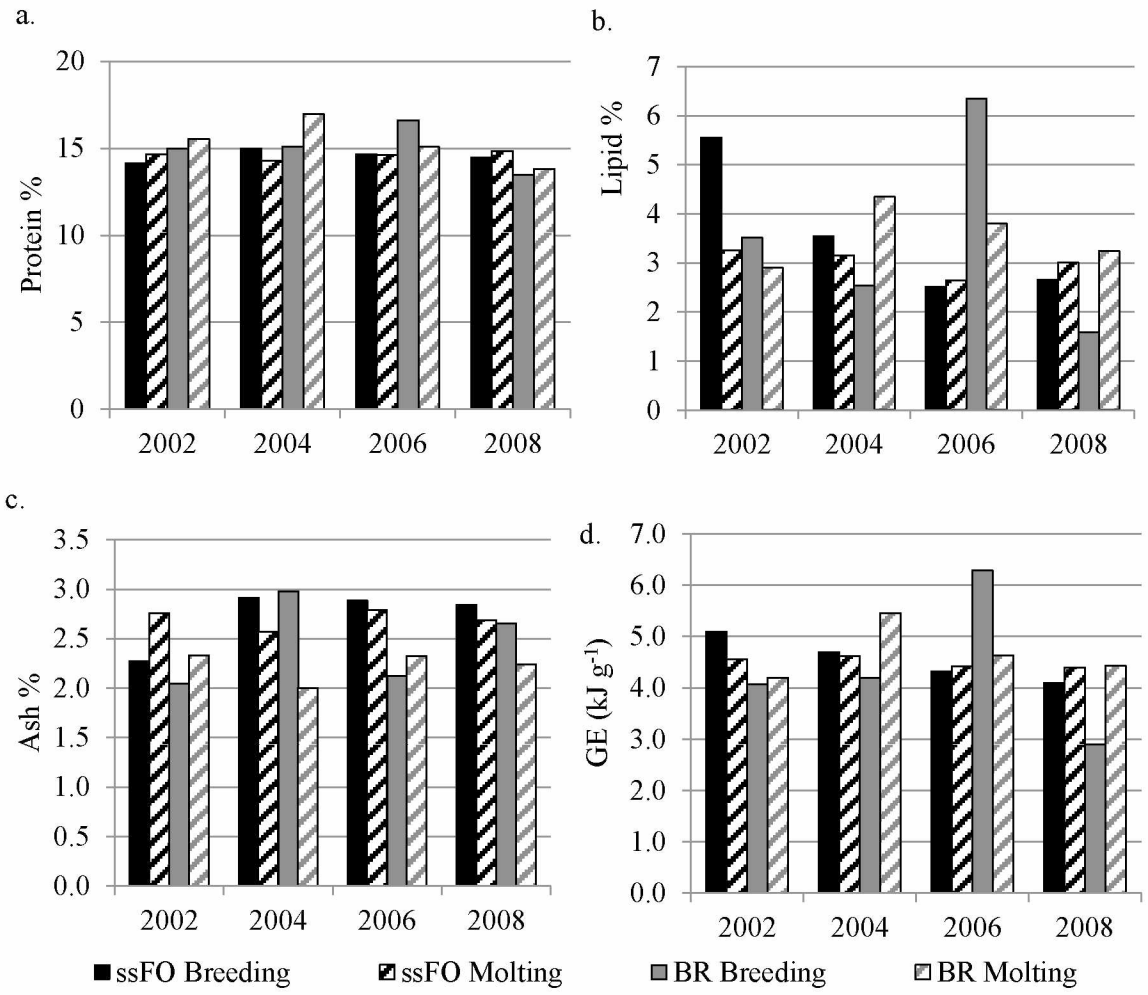

Figure 1.6: Predicted nutrient composition of breeding and molting season harbor seal (*Phoca vitulina*) diets. Estimated diets calculated using split-sample frequency of occurrence (ssFO) and biomass (BR) calculations from scats collected during the breeding season (May - early July) and the molting season (late July - September). Estimated prey contribution to diet was entered into the MIXIT-WIN program (Agricultural Software Consultants Inc., San Diego, California) to acquire nutritional parameters for each of the diets on a wet-weight basis; a) protein content (%), b) lipid content (%), c) ash content (%), and d) gross energy (GE) content (kJ g^{-1}).

TABLES

Table 1.1: Number of harbor seal (*Phoca vitulina*) scats containing identifiable prey items collected during each collection year during the breeding (May to early-July) and molting (late-July through September) seasons.

Year	Season	*N*
2001	Molting	17
2002	Breeding	18
	Molting	128
2003	Molting	76
2004	Breeding	9
	Molting	47
2005	Molting	42
2006	Breeding	15
	Molting	84
2007	Molting	72
2008	Breeding	52
	Molting	83
2009	Molting	65

Table 1.2: List of all prey species identified in harbor seal (*Phoca vitulina*) scats. Scats collected from Tugidak Island Alaska from 2001 – 2009. Frequency of occurrence (FO) of each species for all years is shown. Contribution to diet was calculated using split-sample frequency of occurrence (ssFO) and biomass (BR) reconstruction estimations. The mean and standard deviation (SD) of prey species relative importance to the diet during the molting period across all the collection years is shown only for prey species with ≥10% occurrence in diet for at least one collection period in the time series (e.g., 2002 molting period). The category provided for each of these species denotes whether the relative importance of each species was estimated to be higher by ssFO calculations than BR (category 1), higher according to BR than ssFO (category 2), or if neither calculation method dominated (category 3). Examples of each category are portrayed in Figure 1.2.

Species Category	Scientific Name	FO	ssFO Mean ± SD	BR Mean ± SD	Category
Bathymaster					
Ronquil		0.0212	0.72 ± 0.87	0.01 ± 0.03	1
Northern Ronquil	*Ronquilus jordani*				
Searcher		0.1201	3.36 ± 2.40	2.12 ± 1.83	3
Searcher	*Bathymaster signatus*				
Capelin	*Mallotus villosus*	0.0141	1.63 ± 4.48	0.02 ± 0.04	1
Cephalopod		0.1963	8.23 ± 4.22	18.16 ± 13.65	2
Giant Pacific Octopus	*Enteroctopus dofleini*				
Squid					
Eelpout	*Zoarchidae* spp.	0.0014			
Eulachon	*Thaleichthys pacificus*	0.0141	1.04 ± 3.11	0.04 ± 0.11	1
Flatfish					
Arrowtooth Flounder	*Atheresthes stomias*	0.0480	4.26 ± 4.95	2.32 ± 2.95	1
Dover Sole	*Microstomus pacificus*	0.0438	2.94 ± 3.65	1.53 ± 2.59	3
Flathead Sole	*Hippoglossoides elassodon*	0.0099			
Pacific Halibut	*Hippoglossus stenolepis*	0.1271	5.16 ± 3.27	5.06 ± 3.77	3
Petrale Sole	*Eopsetta jordani*	0.0042			
Rex Sole	*Glyptocephalus zachirus*	0.0127	1.10 ± 1.37	0.64 ± 0.67	3
Rock Sole	*Lepidopsetta* spp.	0.2401	8.64 ± 4.12	3.40 ± 2.07	1
Sand Sole	*Psettichthys melanostictus*	0.0042			
Slender Sole	*Lyopsetta exilis*	0.0014			
Starry Flounder	*Platichthys stellatus*	0.0071			

Table 1.2 Continued

Species Category	Scientific Name
Gadid	
Pacific Cod	*Gadus macrocephalus*
Pollock	*Theragra chalcogramma*
Tomcod	*Microgadus proximus*
Gunnel	
Banded Gunnel	*Pholis fasciata*
Penpoint Gunnel	*Apodichthys flavidus*
Herring	*Clupea pallasi*
Hexagrammids	
Atka Mackerel	*Pleurogrammus monopterygius*
Greenling	
Kelp Greenling	*Hexagrammos decagrammus*
Lingcod	*Ophiodon elongates*
Poacher	*Agonidae* spp.
Prowfish	*Zaprora silensus*
Rockfish	*Sebastes* spp.
Sablefish	*Anoplopoma fimbria*
Salmon	
Chinook Salmon	*Oncorhynchus tshawytscha*
Coho Salmon	*Oncorhynchus kisutch*
Chum Salmon	*Oncorhynchus keta*
Pink Salmon	*Oncorhynchus gorbuscha*
Sockeye Salmon	*Oncorhynchus nerka*
Sand fish	*Trichodon trichodon*
Sand lance	*Ammodytes hexapterus*

FO	ssFO Mean ± SD	BR Mean ± SD	Category
0.1879	6.17 ± 3.42	8.47 ± 9.39	3
0.0184	1.08 ± 1.21	0.65 ± 0.86	3
0.0014			
0.0254	1.23 ± 1.39	0.13 ± 0.19	1
0.0071			
0.2161	11.40 ± 4.63	10.08 ± 4.70	3
0.0056			
0.0226	0.83 ± 1.11	0.14 ± 0.20	1
0.0042			
0.0127			
0.0028			
0.0876	7.87 ± 6.59	30.18 ± 17.43	2
0.0240	1.05 ± 1.00	0.20 ± 0.28	1
0.0946	2.05 ± 2.43	0.58 ± 0.85	1

Table 1.2 Continued

Species Category	Scientific Name
Scuplin	
Enophrys spp.	
Antlered Sculpin	*Enophrys diceraus*
Buffalo Sculpin	*Enophrys bison*
Leister Sculpin	*Enophrys lucasi*
Gymnocanthus spp.	
Armorhead Sculpin	*Gymnocanthus galeatus*
Irish Lord	
Brown Irish Lord	*Hemilepidotus spinosus*
Red Irish Lord	*Hemilepidotus hemilepidotus*
Yellow Irish Lord	*Hemilepidotus jordani*
Triglops spp.	
Ribbed Sculpin	*Triglops pingelii*
Roughspine Sculpin	*Triglops macellus*
Misc.	
Bigmouth Sculpin	*Hemitripterus bolini*
Butterfly Sculpin	*Hemilepidotus papilio*
Great-Type Sculpin	*Myoxocephalus* spp.
Padded Sculpin	*Artedius fenestralis*
Plain Sculpin	*Myoxocephalus jaok*
Spinyhead Sculpin	*Dasycottus setiger*
Staghorn Sculpin	*Leptocottus armatus*
Warty Sculpin	*Myoxocephalus verrucosus*
Snailfish	*Liparidinae* spp.

FO	ssFO Mean ± SD	BR Mean ± SD	Category
0.0014			
0.0240			
0.0014			
0.1059	3.18 ± 2.14	0.97 ± 0.71	1
0.5056	22.38 ± 8.34	14.76 ± 6.63	1
0.1356	5.69 ± 3.41	0.55 ± 0.46	1
0.0014			
0.0014			
0.0056			
0.0141			
0.0014			
0.0042			
0.0028			
0.0042			
0.0297			

Table 1.2

Species Category	Scientific Name
Stichaeidae	
Arctic Shanny	*Stichaeus punctatus*
Warbonnet	
Decorated Warbonnet	*Chirolophis decoratus*
High Cockscomb	*Anoplarchus purpurescens*
Prickleback	
Nutcracker Prickleback	*Bryozoichthys lysimus*
Snake Prickleback	*Lumpenus sagittal*

FO	ssFO Mean ± SD	BR Mean ± SD	Category
0.0014			
0.0071			
0.0071			
0.0014			
0.0042			

LITERATURE CITED

Alverson DL (1991) Commercial fisheries and the Steller sea lion (*Eumetopias jubatus*): The conflict arena. Fisheries Research Institute, School of Fisheries, University of Washington, Seattle, WA, p99

Andersen SM, Lydersen C, Grahl-Nielsen O, Kovacs KM (2004) Autumn diet of harbour seals (*Phoca vitulina*) at Prins Karls Forland, Svalbard, assessed via scat and fatty-acid analyses. Canadian Journal of Zoology 82:1230-1245

Anderson P, Piatt J (1999) Community reorganization in the Gulf of Alaska following ocean climate regime shift. Marine Ecology Progress Series 189:117-123

Antonelis G, Sinclair E, Ream R, Robson B (1997) Inter-island variation in the diet of female northern fur seals (*Callorhinus ursinus*) in the Bering Sea. Journal of Zoology 242:435-451

Arim M, Naya DE (2003) Pinniped diets inferred from scats: analysis of biases in prey occurrence. Canadian Journal of Zoology 81:67-73

Beamish RJ, Bouillon DR (1993) Pacific salmon production trends in relation to climate. Canadian Journal of Fisheries and Aquatic Sciences 50:1002-1016

Berg I, Haug T, Nilssen KT (2002) Harbour seal (*Phoca vitulina*) diet in Vesterålen, North Norway. Sarsia - North Atlantic Marine Science 87:451-461

Boness D, Bowen W, Oftedall O (1994) Evidence of a maternal foraging cycle resembling that of otariid seals in a small phocid, the harbor seal. Behavioral Ecology and Sociobiology 34:95-104

Boness DJ, Bowen WD (1996) The evolution of maternal care in pinnipeds. Bioscience 46:645-654

Boness DJ, Bowen WD, Buhleier BM, Marshall GJ (2006) Mating tactics and mating system of an aquatic-mating pinniped: the harbor seal, *Phoca vitulina*. Behavioral Ecology and Sociobiology 61:119-130

Bowen W, Boness D, Iverson S (1999) Diving behaviour of lactating harbour seals and their pups during maternal foraging trips. Canadian Journal of Zoology 77:978-988

Bowen W (2000) Reconstruction of pinniped diets: accounting for complete digestion of otoliths and cephalopod beaks. Canadian Journal of Fisheries and Aquatic Sciences 57:898-905

Bowen W, Iverson S, Boness D, Oftedal OT (2001) Foraging effort, food intake and lactation performance depend on maternal mass in a small phocid seal. Functional Ecology 15:325-334

Burns JJ (2009) Harbor seal and spotted seal. In: Perrin WF, Würsig B, Thewissen JGM (eds) Encyclopedia of Marine Mammals. Academic Press, San Francisco

Casper R, Gales N, Hindell M, Robinson S (2006) Diet estimation based on an integrated mixed prey feeding experiment using *Arctocephalus* seals. Journal of Experimental Marine Biology and Ecology 328:228-239

Clarke MR (1962) The identification of cephalopod" beaks" and the relationship between beak size and total body weight. Bulletin of the British Museum (Natural History) Zoology 8 (10):419-480

Clarke MR (1986) A handbook for the identification of cephalopod beaks. Oxford University Press, New York,

Coltman DW, Bowen W, Boness DJ, Iverson SJ (1997) Balancing foraging and reproduction in the male harbour seal, an aquatically mating pinniped. Animal Behaviour 54:663-678

Conners ME, Conrath C (2010) Assessment of the octopus complex in the Gulf of Alaska. Alaska Fisheries Science Center, North Pacific Fisheries Management Council Gulf of Alaska SAFE Report, p745-776

Cottrell P, Trites A, Miller E (1996) Assessing the use of hard parts in faeces to identify harbour seal prey: results of captive-feeding trials. Canadian Journal of Zoology 74:875-880

Crowley PH (1992) Resampling methods for computation-intensive data analysis in ecology and evolution. Annual Review of Ecology and Systematics 23:405-447

da Silva J, Neilson JD (1985) Limitations of using otoliths recovered in scats to estimate prey consumption in seals. Canadian Journal of Fisheries and Aquatic Sciences 42:1439-1442

Daniel RG, Jemison LA, Pendleton GW, Crowley SM (2003) Molting phenology of harbor seals on Tugidak Island, Alaska. Marine Mammal Science 19:128-140

Dehn L-A, Sheffield GG, Follmann EH, Duffy LK, Thomas DL, O'Hara TM (2007) Feeding ecology of phocid seals and some walrus in the Alaskan and Canadian Arctic as determined by stomach contents and stable isotope analysis. Polar Biology 30:167-181

Gende S, Quinn T, Willson M (2001) Consumption choice by bears feeding on salmon. Oecologia 127:372-382

Gende SM, Quinn TP, Willson MF, Heintz R, Scott TM (2004) Magnitude and fate of salmon-derived nutrients and energy in a coastal stream ecosystem. Journal of Freshwater Ecology 19:149-160

Grigg EK, Klimley AP, Allen SG, Green DE, Elliott-Fisk DL, Markowitz H (2009) Spatial and seasonal relationships between Pacific harbor seals (*Phoca vitulina richardii*) and their prey, at multiple scales. Fishery Bulletin 107:359-372

Hammond P, Rothery P (1996) Application of computer sampling in the estimation of seal diet. Journal of Applied Statistics 23:525-534

Harvey J (1989) Assessment of errors associated with harbour seal (*Phoca vitulina*) faecal sampling. Journal of Zoology 219:101-111

Hauser DDW, Allen CS, Rich HB, Quinn TP (2008) Resident harbor seals (*Phoca vitulina*) in Iliamna Lake, Alaska: summer diet and partial consumption of adult sockeye salmon (*Oncorhynchus nerka*). Aquatic Mammals 34:303-309

Herreman JK, Blundell GM, Ben-David M (2009) Evidence of bottom-up control of diet driven by top-down processes in a declining harbor seal *Phoca vitulina richardsi* population. Marine Ecology Progress Series 374:287-300

Huber HR, Jorgensen JC, Butler VL, Baker G, Stevens R (2011) Can salmonids (*Oncorhynchus* spp.) be identified to species using vertebral morphometrics? Journal of Archaeological Science 38:136-146

Jackson J, Dinnocenzo J (2012) Kodiak management area harvest strategy for the 2012 commercial salmon fishery. Alaska Department of Fish and Game, Anchorage, p64

Jemison L (2001) Summary of harbor seal diet data collected in Alaska from 1990-1999. Alaska Department of Fish and Game, Division of Wildlife Conservation, Anchorage, AK, p314-322

Jemison LA, Kelly BP (2001) Pupping phenology and demography of harbor seals (*Phoca vitulina richardii*) on Tugidak Island, Alaska. Marine Mammal Science 17:585-600

Jemison L, Pendleton G, Wilson C, Small R (2006) Long-term trends in harbor seal numbers at Tugidak Island and Nanvak Bay, Alaska. Marine Mammal Science 22:339-360

Kitts D, Huynh M, Hu C, Trites A (2004) Season variation in nutrient composition of Alaskan walleye pollock. Canadian Journal of Zoology 82:1408-1415

Klare U, Kamler JF, MacDonald DW (2011) A comparison and critique of different scat analysis methods for determining carnivore diet. Mammal Review 41:294-312

Laake J, Browne P, DeLong R, Huber H (2002) Pinniped diet composition: a comparison of estimation models. Fish Bulletin 100:434-447

Lance MM, Jeffries SJ (2007) Temporal and spatial variability of harbor seal diet in the San Juan Island archipelago. SeaDoc Society, Washington Department of Fish and Wildlife, Lakewood, WA, p29

London JM, Lance MM, Jeffries SJ (2002) Observations of harbor seal predation on Hood Canal salmonids from 1998 to 2000. NOAA Grant No. NA17FX1603, WDFW, PSMFC, p20

Mantua NJ, Hare SR (2002) The Pacific decadal oscillation. Journal of Oceanography 58:35-44

Mathisen OA, Lopp RJ (1963) Photographic census of the Steller sea lion herds in Alaska, 1956-1958. United States Fish and Wildlife Service, Special Scientific Report of Fisheries 424

McGowan JA, Cayan DR, Dorman LRM (1998) Climate-ocean variability and ecosystem response in the Northeast Pacific. Science 281:210

McKenzie J, Wynne KM (2008) Spatial and temporal variation in the diet of Steller sea lions in the Kodiak Archipelago, 1999 to 2005. Marine Ecology Progress Series 360:265-283

Merrick RL, Chumbley MK, Byrd GV (1997) Diet diversity of Steller sea lions (*Eumetopias jubatus*) and their population decline in Alaska: a potential relationship. Canadian Journal of Fisheries and Aquatic Sciences 54:1342-1348

Middlemas SJ, Barton TR, Armstrong JD, Thompson PM (2006) Functional and aggregative responses of harbour seals to changes in salmonid abundance. Proceedings: Biological Sciences 273:193-198

Mueter FJ, Ware DM, Peterman RM (2002) Spatial correlation patterns in coastal environmental variables and survival rates of salmon in the north-east Pacific Ocean. Fisheries Oceanography 11:205-218

Olesiuk PF, Bigg MA, Ellis GM, Crockford SJ, Wigen RJ (1990) An assessment of the feeding habits of harbour seals (*Phoca vitulina*) in the Strait of Georgia, British Columbia, based on scat analysis. Canadian Technical Report of Fisheries and Aquatic Science 1730:135

Orchard TJ (2003) An application of the linear regression technique for determining length and weight of six fish taxa: the role of selected fish species in Aleut paleodiet. Archaeopress Oxford, United Kingdom

Orr AJ, Harvey JT (2001) Quantifying errors associated with using fecal samples to determine the diet of the California sea lion (*Zalophus californianus*). Canadian Journal of Zoology 79:1080-1087

Page B, McKenzie J, Goldsworthy SD (2005) Dietary resource partitioning among sympatric New Zealand and Australian fur seals. Marine Ecology Progress Series 293:283-302

Phillips EM, Harvey JT (2009) A captive feeding study with the Pacific harbor seal (*Phoca vitulina richardii*): Implications for scat analysis. Marine Mammal Science 25:373-391

Pitcher K (1980) Stomach contents and feces as indicators of harbor seal, *Phoca vitulina*, foods in the Gulf of Alaska. Fishery Bulletin 78:797-798

Pitcher KW (1981) Prey of the Steller sea lion, *Eumetopias jubatus*, in the Gulf of Alaska. Fisheries Bulletin (Seattle) 79:467-472

Pitcher KW (1990) Major decline in number of harbor seals, *Phoca vitulina richardsi*, on Tugidak Island, Gulf of Alaska. Marine Mammal Science 6:121-134

Prime J (1979) Observations on the digestion of some gadoid fish otoliths by a young common seal. International Council for the Exploration of the Seas Conference and Meeting Documents:1-3

Ray GC, McCormick-Ray J, Berg P, Epstein HE (2006) Pacific walrus: benthic bioturbator of Beringia. Journal of Experimental Marine Biology and Ecology 330:403-419

Robards MD, Anthony JA, Rose GA, Piatt JF (1999) Changes in proximate composition and somatic energy content for Pacific sand lance (*Ammodytes hexapterus*) from Kachemak Bay, Alaska relative to maturity and season. Journal of Experimental Marine Biology and Ecology 242:245-258

Robinson SMC, Hartwick EB (1983) The relationship between beak morphometrics and the live wet weight of the giant Pacific octopus, *Octopus dofleini martini* (Wülker). The Veliger 26:26-29

Rosen D, Trites A (2000) Pollock and the decline of Steller sea lions: testing the junk-food hypothesis. Canadian Journal of Zoology 78:1243-1250

Shannon CEW (1949) The mathematical theory of communication. Urbana, Univ of Illinois Press

Sigler M, Tollit D, Vollenweider J, Thedinga J, Csepp D, Womble J, Wong M, Rehberg M, Trites A (2009) Steller sea lion foraging response to seasonal changes in prey availability. Marine Ecology Progress Series 388:243-261

Sinclair E, Zeppelin T (2002) Seasonal and spatial differences in diet in the western stock of Steller sea lions (*Eumetopias jubatus*). Alaska Fisheries Science Center, National Marine Fisheries Service, NOAA, p1-42

Small R, Pendleton G, Pitcher K (2003) Trends in abundance of Alaska harbor seals, 1983-2001. Marine Mammal Science 19:344-362

Small RJ, Lowry LF, Hoef JM, Frost KJ, Delong RA, Rehberg MJ (2005) Differential movements by harbor seal pups in contrasting Alaska environments. Marine Mammal Science 21:671-694

Smith TM, Reynolds RW, Peterson TC, Lawrimore J (2008) Improvements to NOAA's historical merged land-ocean surface temperature analysis (1880-2006). Journal of Climate 21:2283-2296

Southward GM, Hardman WH (1973) Otolith length and fish length of Pacific halibut. International Pacific Halibut Commission, p10

Stanberry K (2003) The effect of changes in dietary fat level on body composition, blood metabolites and hormones, rate of passage, and nutrient assimilation efficiency in harbor seals. Master of Science, University of Hawaii at Manoa, Honolulu, HI, p116

Thompson P, Fedak M, McConnell B, Nicholas K (1989) Seasonal and sex-related variation in the activity patterns of common seals (*Phoca vitulina*). Journal of Applied Ecology 26:521-535

Thompson PM, Miller D, Cooper R, Hammond PS (1994) Changes in the distribution and activity of female harbour seals during the breeding season: implications for their lactation strategy and mating patterns. Journal of Animal Ecology 63:24-30

Tollit D, Steward M, Thompson P, Pierce G, Santos M, Hughes S (1997) Species and size differences in the digestion of otoliths and beaks: implications for estimates of pinniped diet composition. Canadian Journal of Fisheries and Aquatic Sciences 54:105-119

Tollit D, Black A, Thompson P, Mackay A, Corpe H, Wilson B, Parijs S, Grellier K, Parlane S (1998) Variations in harbour seal *Phoca vitulina* diet and dive-depths in relation to foraging habitat. Journal of Zoology 244:209-222

Tollit D, Wong M, Winship A, Rosen D, Trites A (2003) Quantifying errors associated with using prey skeletal structures from fecal samples to determine the diet of Steller's sea lion (*Eumetopias jubatus*). Marine Mammal Science 19:724-744

Tollit D, Heaslip S, Deagle B, Iverson S, Joy R, Rosen D, Trites A (2006) Estimating diet composition in sea lions: which technique to choose? In: Trites A, Atkinson S, DeMaster D, Fritz L, Gelatt T, Rea L, Wynne K (eds) Sea lions of the world. Alaska Sea Grant College Program, University of Alaska, Fairbanks, AK, p293–308

Trites AW, Donnelly CP (2003) The decline of Steller sea lions *Eumetopias jubatus* in Alaska: a review of the nutritional stress hypothesis. Mammal Review 33:3-28

Trites AW, Joy R, Weckerly FW (2005) Dietary analysis from fecal samples: How many scats are enough? Journal of Mammalogy 86:704-712

Trites AW, Calkins DG, Winship AJ (2007) Diets of Steller sea lions (*Eumetopias jubatus*) in Southeast Alaska, 1933-1999. Fishery Bulletin 105:234-248

Trumble SJ, Barboza PS, Castellini MA (2003) Digestive constraints on an aquatic carnivore: effects of feeding frequency and prey composition on harbor seals. Journal of Comparative Physiology B: Biochemical, Systemic, & Environmental Physiology 173:501-509

Trumble SJ, Castellini MA (2005) Diet mixing in an aquatic carnivore, the harbour seal. Canadian Journal of Zoology 83:851-859

Vollenweider JJ, Heintz RA, Schaufler L, Bradshaw R (2011) Seasonal cycles in whole-body proximate composition and energy content of forage fish vary with water depth. Marine Biology 158:413-427

Waite J, Burkanov V, Andrews R (2012) Prey competition between sympatric Steller sea lions (*Eumetopias jubatus*) and northern fur seals (*Callorhinus ursinus*) on Lovushki Island, Russia. Canadian Journal of Zoology 90:110-127

Wilcoxon F (1945) Individual comparisons by ranking methods. Biometrics Bulletin 1:80-83

Wright BE, Riemer SD, Brown RF, Ougzin AM, Bucklin KA (2007) Assessment of harbor seal predation on adult salmonids in a Pacific Northwest estuary. Ecological Applications 17:338-351

Zhao L, Castellini MA, Schell DM (2006) Metabolic adjustments to varying protein intake in harbor seals (*Phoca vitulina*): evidence from serum free amino acids. Physiological & Biochemical Zoology 79:965-976

CHAPTER 2: Identification of sex and age class from fecal corticosteroid metabolites in free-ranging harbor seals (*Phoca vitulina*) and application to dietary studies[9]

ABSTRACT

Alterations to a predator's prey base may have substantial impacts on population success. Fecal analysis is a common method to evaluate diet but determining sex and age class of the animal that deposited it can be challenging. In many different species corticosteroid metabolites deposited in feces ("scats") are metabolized differently by age classes and sexes, allowing for the separation of these groups based on their steroid metabolism. The goal of this study was to identify harbor seal sex and age class from scats and to compare diets consumed by these different groups. Scats were collected from free-ranging harbor seals (*Phoca vitulina*) on Tugidak Island, Alaska in summer 2003 and from male and female captive harbor seals. Immunoreactive hormone profiles identified two distinct patterns of corticosteroid metabolites. Immunoreactive profiles from a known adult male differed from known adult female and juvenile male seals. These profiles were compared to wild scats of unknown origin to assess age/sex differences. Diversity of diets consumed by the group with hormone profiles similar to known adult females and/or juveniles was significantly higher than for the group with profiles similar to the known adult male. However, nutritional quality (based on proximate composition) of the diets was not significantly different among groups, indicating that harbor seals of different sex or age classes may have different diets but with similar nutritional value.

[9] Geiger, G.L., S. Atkinson, G.M. Blundell, J.R. Carpenter, K. Mashburn, J. Waite, and K. Wynne. 2012. Formatted in accordance with requirements for Marine Ecology Progress Series.

INTRODUCTION

Harbor seals (*Phoca vitulina*) are small phocids with wide ranging distribution, covering much of the northern hemisphere (Reeves et al. 2002, Burns 2009). The subspecies *Phoca vitulina richardii* is found in the Pacific Ocean extending from the Aleutian Islands in Alaska to Baja California, Mexico (Reeves et al. 2002, Burns 2009). Counts of harbor seals hauled out at Tugidak Island, Alaska during the 1950s suggested that this haul-out may have been the site of some of the highest concentrations of harbor seals in the world (Pitcher 1990) with nearly 17,000 seals being tallied (Mathisen & Lopp 1963). However, starting in the mid-1970s, pinniped species throughout Alaska, including the harbor seals at Tugidak Island, declined precipitously (Pitcher 1990, Jemison et al. 2006). The population of harbor seals at Tugidak Island declined by an estimated 85% between 1976 and 1988 with a reduction of approximately 19% per year between 1976 and 1982 (Pitcher 1990). Though the population has since stabilized, and exhibited a moderate increase of about 6.6% per year between 1993 and 2001 (Small et al. 2003), the population still remains far reduced compared to its historic population size (Jemison et al. 2006).

The reasons behind this decline are still poorly understood. One proposed theory for other pinnipeds, the "nutritional stress hypothesis", states that a change in a predator's diet due to changes in prey abundance or prey quality could have led to nutritional stress and subsequently contributed to population declines (Alverson 1991, Rosen & Trites 2000, Trites & Donnelly 2003). Changes in a predator's prey base may either be natural (e.g., related to climate change) or anthropogenic (e.g., increased competition with fisheries for limited resources). The premise is that, even if prey is abundant but is not of sufficient nutritional quality to support the needs of the predator, the overall health and survival of the predator may suffer (Kirsch et al. 2000, Rosen & Trites 2000).

While there are many ways in which prey availability or quality can change, diet and nutritional demand may also vary between sexes or among different age classes. A variety of factors, including reproductive status, diving capabilities (Page et al. 2005), and strategies to maximize fitness (Thompson et al. 1989, Bleich et al. 1997, Le Boeuf et al. 2000, Beck et al. 2007) can cause one sex or age class to be more impacted by changes to the prey base than another. Even though harbor seals are not sexually dimorphic and do not demonstrate different diving performances (Hastings et al. 2004), sex or age specific behaviors during the breeding and molting periods could result in different foraging strategies and diets. Unlike many other phocids, which will fast throughout the entire lactation period, harbor seal females usually resume foraging during their approximately 24 day long lactation period (Bowen et al. 1992, Boness et al. 1994, Boness & Bowen 1996, Bowen et al. 1999). Because the female is still caring for a dependent pup, her ability to access distant foraging sites may be restricted (Thompson et al. 1994), and she may have to forage on less nutritionally profitable prey. Likewise, males also may restrict their foraging ranges during the breeding season. Males may reduce the amount of offshore foraging to increase their chances of encountering females coming and going from haul-out sites where their pups are located (Coltman et al. 1997, Boness et al. 2006). Therefore, during the breeding season, which occurs between May and early July on Tugidak Island (Jemison & Kelly 2001), breeding requirements of both male and female harbor seal may lead to narrowed foraging ranges.

A common method for analyzing diet composition in free-ranging pinniped populations is analysis of prey remains found in scats. It is relatively inexpensive, non-invasive, and can yield relatively large sample sizes (Pitcher 1980, Cottrell et al. 1996, Merrick et al. 1997, Berg et al. 2002, Andersen et al. 2004, Casper et al. 2006, Tollit et al. 2006). However, scats are not easily attributable to an individual seal and therefore the sex or age of the animal is generally unknown. Consequently, development of a method to identify sex or age class from deposited scats would

increase the usefulness of hard-part analysis of scats. A method to identify the sex of Steller sea lions (*Eumetopias jubatus*) from fecal corticosterone profiles in scats (Mashburn & Atkinson 2004) was developed using the metabolism of the dominant glucocorticoid. Corticosterone is a steroid hormone in a class of hormones known as the glucocorticoids, which are produced in the adrenal cortex (Norman & Litwack 1997, Norris 1997, Kacsoh 2000, von der Ohe & Servheen 2002, Palme et al. 2005, Touma & Palme 2005). Glucocorticoids, including corticosterone and cortisol, are major metabolic hormones (Norman & Litwack 1997, Norris 1997, Kacsoh 2000, Nelson 2000, von der Ohe & Servheen 2002) and are secreted in normal circadian and seasonal rhythms in many species (Norris 1997, Möstl & Palme 2002, Huber et al. 2003, Oki & Atkinson 2004, Keay et al. 2006, Landys et al. 2006). Elevated levels of corticoserone and cortisol are also common indicators of stress responses in pinnipeds (Mashburn & Atkinson 2004, Touma & Palme 2005, Keay et al. 2006, Mashburn & Atkinson 2008).

Before excretion, glucocorticoids are heavily metabolized, and the original parent hormone may only be present in minute quantities in scat (Goymann et al. 2002, Baltic et al. 2005, Möstl et al. 2005, Palme et al. 2005, Rolland et al. 2005, Touma & Palme 2005, Schwarzenberger 2007). The metabolism of corticosterone into its various metabolites is shown in Figure 2.1 (Norman & Litwack 1997). Pronounced differences in the dominant glucocorticoid metabolites identified in scats occur among different species and between the sexes (Wasser et al. 2000, von der Ohe & Servheen 2002, Touma et al. 2003, Mashburn & Atkinson 2004, Rettenbacher et al. 2004, Baltic et al. 2005, Möstl et al. 2005, Palme et al. 2005, Touma & Palme 2005, Keay et al. 2006, Mashburn & Atkinson 2007) and also potentially between age groups (i.e., juvenile and adult) (Mashburn & Atkinson 2008). Determining animal sex by identifying corticosteroid metabolite profiles in feces has previously been demonstrated in mice (*Mus musculus* f. *domesticus*) (Touma et al. 2003), black grouse (*Tetrao tetrix*) (Baltic et al. 2005),

chickens (ISA Brown, hybrid) (Rettenbacher et al. 2004), and adult Steller sea lions (Mashburn & Atkinson 2004).

The objectives of this study were to 1) validate the use of fecal corticosterone metabolite profiles as a means of identifying seal sex and/or age class by contrasting profiles obtained from scats of known sex and age classes (i.e., adult and juvenile) of harbor seals, 2) identify sex and/or age class of unknown harbor seals from scat samples collected from Tugidak Island during the molting period in 2003, and 3) compare prey composition and nutritional value of diets of potentially different sexes and/or age classes of harbor seals from Tugidak Island during the molting period in 2003. We hypothesized that male and female harbor seals would yield different corticosterone metabolite profiles and that comparable profiles would be obtained from scats collected from unidentified, free-ranging harbor seals from Tugidak Island. We further hypothesized that, following separation of the diets of the unknown samples into sex and/or age classes, the diets of juvenile and adult male and female harbor seals would differ in terms of prey composition and nutritional quality.

METHODS

Sample Collection and Processing

One scat was obtained from each of five captive harbor seals of known sex and age housed at the Alaska Zoo in Anchorage, Alaska (1 adult female and 1 juvenile male) and the Alaska SeaLife Center in Seward, Alaska (2 adult females and 1 adult male). Scat samples from harbor seals housed at the Alaska SeaLife Center were obtained from archived samples, while scats from the Alaska Zoo harbor seals were collected opportunistically as part of a husbandry effort. All animals were held individually so that scats could be attributed to the individual seal. Scats were frozen until further processing (Stanberry 2003). Because only one scat could be

obtained from each animal, and only one adult male and one juvenile harbor seal were able to be sampled, there is potential for variation in these profiles that will not be accounted for in this study.

Scats (n=16) from free-ranging harbor seals of unknown sex and age class were collected by the Alaska Department of Fish and Game (ADF&G) from two haul-outs on Tugidak Island, Alaska (Figure 2.2). Collections occurred on August 2 (n=10) and September 6 (n=6), 2003 during the molting period of harbor seals on Tugidak Island (Daniel et al. 2003). The scats used in this analysis all had identifiable prey hard-parts and identified corticosteroid metabolite profiles so these profiles could be matched to diet. Scats were stored frozen at -20°C in individual ziplock bags until further processing. Free-ranging harbor seal scat samples were collected under NMFS permit numbers 358-2585 and 358-1787.

After thawing each scat, a subsample (~2-5 g) of each scat was isolated. As prey hard-parts needed to be protected from damage, loosely bound scats were homogenized by shaking the bag they were held in and subsampled after mixing. Scats that were densely packed had small chunks broken off from multiple areas of the scat to increase homogeneity and to minimize the potential for uneven distribution of hormones in scat. The subsample was placed in a new, individually labeled ziplock bag and frozen at -20°C for future hormone extraction. The remaining scat was washed through a set of nested sieves (1000 μm, 710 μm, 500 μm) with the aid of a dissolution agent (dish soap) to break up the scat so prey remains could be separated without damage. Prey hard-parts were dried, then placed in individually labeled ziplock bags, and sent to Pacific IDentifications Inc. (PacID; British Columbia, Canada) to be identified to the lowest possible taxonomic level using their extensive reference collection.

Steroid Hormone Extraction

Steroid hormones were extracted from scat subsamples following previously published protocols, modified for harbor seal samples (Monfort et al. 1998, Mashburn & Atkinson 2004). All scat samples were homogenized and dried. Approximately 0.025 g of dried fecal powder was weighed to extract steroid hormones (Mashburn & Atkinson 2004). In cases where the subsamples did not exhibit any immunoreactivity, the concentration of steroid hormones was assumed to be too low for detection and these samples were re-extracted using approximately 0.25 g of dried fecal powder (Monfort et al. 1998) to increase the amount of steroid hormones. To extract steroid hormones, dried scat subsamples were boiled in 8ml of 100% ethanol for 20 min, using a water-bath on a hotplate to ensure even heating. Prior to boiling, 10 µl of ^{3}H-corticosterone was added to each sample to assess percent recovery of corticosterone through the extraction procedure. Following boiling, this mixture was centrifuged for 15 min at 1500 rpm and the supernatant was collected in a clean, glass tube and allowed to dry in a fume hood. After drying, the tube walls were rinsed with 100% ethanol (4 ml) and again dried. One ml of 100% methanol was used to reconstitute the extract. It was transferred to plastic test tubes, dried down, and kept frozen at -20°C until further analysis.

High-Performance Liquid Chromatography

High-performance liquid chromatography (HPLC; Waters, Milford, Massachusetts) was used to separate corticosterone metabolites in the scats. Dried fecal steroid extracts were prepared for HPLC by reconstitution in 1 ml phosphate-buffered saline (PBS; pH 5.0) and vortexing (~1 min). A tissue tearer was used against the outside of the tube to agitate the contents and dislodge extract and steroids from the tube walls. The samples were pre-processed using C-18 Sep-Pak cartridges (Waters, Milford, Massachusetts). A 50 µl aliquot of the eluant was injected into the

HPLC and separated through a reverse-phased, silica based C18 column (Waters Spherisorb 5 μm ODS1, 4.6 x 250 mm; Waters, Milford, Massachusetts) with a linear gradient of 20-100% methanol in filtered, deionized water over 80 min at a flow rate of 1 ml/min. The gradient separated corticosteroid metabolites into the fractions based on the polarity of the metabolites. One fraction (1 ml) was collected every minute for 80 min and then dried.

Radioimmunoassay

Radioimmunoassay (RIA) is a procedure where a radioactively labeled antigen and highly specific antibody are combined with an unknown sample of standard hormone and the amount of bound antibody is counted as a measure of the concentration of hormone in the sample. In this study, RIA was used to identify the presence of corticosterone metabolites in the fractions recovered from HPLC of harbor seal fecal steroid hormone extracts. A double-antibody ^{125}I-corticosterone RIA (MP Biomedicals, LLC, Solon, Ohio) was validated for use with extracted scats. Assays were performed according to the manufacturer's instructions with the exception that all reagents were halved and an additional standard was included (one half the lowest standard) in an effort to increase detection sensitivity (Mashburn & Atkinson 2004). The concentrations of corticosterone in the standards were 12.5 ng/ml, 25 ng/ml, 50 ng/ml, 100 ng/ml, 250 ng/ml, 500 ng/ml, and 1000 ng/ml. Additional controls were analyzed to account for the total counts of ^{125}I added to each tube, the non-specific binding counts (the amount of ^{125}I corticosterone that binds to molecules that were not corticosterone-specific antibodies), and zero controls (where no known corticosterone standard was added). Fractions from HPLC were reconstituted in 50 μl of assay buffer for RIA. The RIA's cross-reactivity with other steroids, as reported by the manufacturer, were: desoxycorticosterone (0.34%), testosterone (0.10%), cortisol (0.05%), aldosterone (0.03%), progesterone (0.02%), androstenedione (0.01%), and all other steroids tested showed <0.01%

cross-reactivity. Therefore, the cross-reactivity of this RIA with other steroid hormones present in the extract was expected to be minimal.

Validation of HPLC Techniques

HPLC data were validated in two ways: 1) using immunoreactivity profiles of known steroid hormones, corticosterone and cortisol, and 2) using scats from harbor seals of known sex and age class. Tracers of ^{3}H-corticosterone and ^{3}H-cortisol in 100% methanol were injected through the HPLC and 80 fractions were collected to identify the fraction at which these hormones appeared. This was done to determine if the observed peaks in the various fractions of each sample were the parent hormone corticosterone or if cross-reactivity occurred with cortisol, which is a predominant circulating glucocorticoid steroid hormone. Corticosterone standard in serum was also processed using HPLC and RIA of collected fractions as a measure of quality assurance. This was done to evaluate the effect that drag created by a biological medium could have on the fraction in which corticosterone was identified. Scat samples from one adult male, three adult females, and one sub-adult male were processed in the same manner as the unknown harbor seal samples with the following exception. To ensure that immunoreactive profiles from these samples would be obtainable, all samples were initially extracted from 0.25 g of dried scat powder instead of 0.025 g. Fecal corticosterone metabolite profiles were compared between known and unknown sex and age class samples.

Diet Assessment

PacID provided data which included identification of prey species isolated from each scat, confidence codes associated with species identification, the structure used to identify the prey item and its condition, the minimum number of individuals (MNI) of each prey in a sample, and approximate size of the prey in each scat. Scats containing prey items that could not be

confidently identified (i.e., unidentified fish species) were eliminated from further analysis. Cephalopod beaks were individually examined using a combination of identification guides (Clarke 1962, 1986), photographs of beaks of giant Pacific octopus (*Enteroctopus dofleini*; GPO), and knowledge of the species of octopus predominantly found around the Kodiak archipelago within the diving depth limits of harbor seals (Conners & Conrath 2010). Hereafter, the cephalopod category will refer to GPO. Salmon (*Oncorhynchus* spp.) vertebrae width to height ratios were also examined to identify species (Huber et al. 2011). Salmon vertebrae measurements were used to categorize salmon into three groups: 1) Chinook (*O. tshawytscha*), 2) pink (*O. gorbuscha*) or coho (*O. kisutch*), and 3) chum (*O. keta*) or sockeye (*O. nerka*) salmon (Huber et al. 2011). These salmon categories were used for nutritional analysis by calculating the proportion that each species contributed to the average harbor seal diet.

Observed Shannon diversity indices (SDI) were calculated with data returned from PacID using the following equation:

$$H' = -\sum_{i=1}^{S} p_i \, (\ln p_i)$$

where H' is the SDI, p_i is the relative abundance of species i, and S is the number of species identified (Shannon 1949). The SDI is a common index used for estimating diet diversity of pinnipeds (Sinclair & Zeppelin 2002, Trites et al. 2007, McKenzie & Wynne 2008, Herreman et al. 2009, Sigler et al. 2009, Waite et al. 2012). A simple bootstrapping procedure was (Crowley 1992) applied to estimate mean SDI for identified groups and provide 95% confidence intervals so that differences in diet diversity could be evaluated.

Relative dietary importance of prey species was assessed using biomass reconstruction (BR) methods (Tollit et al. 1998, Page et al. 2005, Middlemas et al. 2006, Sigler et al. 2009, Waite et al. 2012). Consumed biomass was estimated using length-weight regression equations

for each prey species from published literature (Appendix 1). The approximate lengths of individual prey were provided by PacID based on size correlations to their reference collection. Minimum number of individuals, provided by PacID, was also used to estimate consumed biomass. Individual measurements of hard parts from Irish lord (*Hemilepidotus* spp.; various bones) (Orchard 2003), GPO (beak morphometrics) (Robinson & Hartwick 1983), and Pacific halibut (*Hippoglossus stenolepis*; otoliths) (Southward & Hardman 1973) were made in an attempt to improve biomass estimations. These species were selected for additional measurement because, 1) equations for calculating body length from length of some bone fragments were available in the published literature, 2) the species had potential to contribute a significant portion to diet (e.g., Irish lord), 3) the species demonstrated large variability in PacID size ranges (e.g., small vs. large halibut), or 4) the species did not have a length estimate assessed (i.e., no estimation of length or weight of GPO provided by PacID). The hard parts were measured using electronic calipers (Model: C20054: Range: 0-150 mm, Resolution: 0.01 mm, Accuracy: ±0.02 mm, Marathon: Richmond Hill, Ontario, Canada). These measurements were expected to provide more accurate estimates of consumed biomass as they were based on measurement of individual bones or beaks, rather than size in comparison to a reference skeleton. The relative importance of each prey species by BR was assumed to be reflected by its percent contribution to the diet.

Nutritional analysis of the average harbor seal diet was performed using the MIXIT-WIN program (version 6.17, 2011; Agricultural Software Consultants Inc., San Diego, California). The MIXIT program was designed to allow agricultural users to construct proposed diets within a financial budget, while still meeting the nutritional requirements determined for the animals. This study used the MIXIT program to evaluate the quality of harbor seal diets, in terms of crude protein, lipid, ash, and gross energy (GE), and compare between sexes and/or age classes (i.e., adult and juvenile). A diet formula was created in the MIXIT program for each individual scat

sample. The nutritional database used for these calculations was compiled from whole-animal proximate composition data in the published literature for prey species from Alaskan waters (Appendix 2). When multiple nutritional data sources were found for a single prey species, the data selected for use in this study were determined first based on sample size and then based on the location where the prey samples were obtained. When available, seasonal variation in prey species proximate composition was taken into account (e.g., sand lance, *Ammodytes hexapterus*, proximate analysis in August compared to September). While prey species sex and reproductive status can influence its nutrient composition, the nutritional data for all prey items in this study are presented on a mixed-sex basis (i.e., proximate composition from male and female prey) as sex and reproductive status of prey consumed were unknown. All values were reported on a wet-weight basis.

Data Analysis

Bootstrapping (Crowley 1992) was used to resample the diet data of the groups identified using corticosteroid metabolite profiles to statistically compare the diet diversity between the groups (R) (Waite et al. 2012). The data were resampled with replacement 1000 times to calculate a mean SDI for the groups. 95% confidence intervals for the SDI were calculated to determine if the diet diversity between sexes and age classes of harbor seals differed. Pairwise differences in nutritional parameters between sexes and age groups were compared using a Wilcoxon signed rank test (Wilcoxon 1945) due to sample size limitations. All statistical analyses were performed with R version 2.15.0 (The R Foundation for Statistical Computing, Vienna, Austria) with $p < 0.05$ considered significant unless otherwise noted.

RESULTS

Steroid Analysis

When HPLC fractions of the corticosterone steroid standard in serum were processed using RIA, an immunoreactive peak was observed at approximately the same fraction as the ^{3}H-corticosterone tracer in 100% methanol (fractions 48-52; ^{3}H-corticosterone tracer: Figure 2.3a; corticosterone steroid standard: Figure 2.3b). This demonstrates that the drag created by a biological medium (i.e., feces) did not impact corticosterone eluting through the column. The cortisol standard showed an immunoreactive peak at fractions 41-42 (Figure 2.3c). A range of fractions where the peak could occur is presented because some variation in the exact fractions of the peak may occur. Knowing the range of fractions the corticosterone and cortisol standards came off the column allowed us to determine whether immunoreactive peaks in the fecal samples were corticosterone or cortisol, or if they represented closely-related metabolites of corticosterone that the RIA kit recognized as being structurally similar to corticosterone.

The known adult male harbor seal sample and the known adult female harbor seal samples expressed different corticosterone metabolite profiles (Figure 2.4). The known adult male profile expressed a peak between fractions 46-52 that co-eluted with the corticosterone standard as well as an immunoreactive peak for an unknown corticosterone metabolite that eluted later than corticosterone, between fractions 55-60 (Figure 2.4a). The known adult female profiles expressed an immunoreactive peak between fractions 46-52 indicative of the corticosterone peak (Figure 2.4b). The known juvenile male also had an immunoreactive peak at the same fractions as corticosterone, between fractions 47-52, but unlike the adult male, the juvenile did not demonstrate the late immunoreactive peak (Figure 2.4c). Therefore, the corticosteroid metabolite profile of the juvenile male was nearly identical to that of the adult females.

Corticosteroid metabolite profiles from scats of free-ranging harbor seals of unknown sex and age class (Figure 2.5) were similar to either the adult male profile (immunoreactive peaks between both fractions 46-52 and between fractions 55-60; n=6) or the profiles of the adult females and the juvenile male (immunoreactive peak only between fractions 46-52; n=10). Samples collected on both collection dates were observed in both groups (adult males: August 2: n=3, September 6: n=3; adult females or juveniles: August 2: n=7, September 6: n=3); therefore, we did not consider collection date to be a confounding factor.

Diet Comparison

The observed SDI for the diets of adult male harbor seals and the combined group containing adult female and juvenile harbor seals was 1.52 and 2.07 (maximum possible SDI=20), respectively. Bootstrapped mean SDIs for each of these groups was lower than their respective observed SDI, but still reflected that diet diversity of adult males was lower than that of adult females and/or juveniles (adult males: 1.41; adult females or juveniles: 1.88). Bootstrapped 95% confidence intervals for pairwise differences in bootstrapped mean SDIs for adult male and adult female and/or juvenile harbor seals did not contain zero (-0.93, -0.17). Therefore, the diet diversity, as determined by the SDI, between the two groups was significantly different ($p < 0.05$) with the diet diversity of adult males being significantly lower than that of the adult females and/or juveniles.

Diets of the presumed adult male harbor seals and the adult females and/or juveniles contained largely the same prey species (Figure 2.6). Prey species consumed by all sexes and age classes included greenling (*Hexagrammid* spp.), rock sole (*Lepidopsetta bilineata*), Pacific cod (*Gadus macrocephalus*), Pacific halibut, GPO, Irish lord, *Triglops* spp., and searcher (*Bathymaster* spp.). In addition, diets consumed by presumed adult females and/or juveniles included salmon, capelin (*Mallotus villosus*) and sand lance. The overall nutritional value of the

diets of presumed adult male harbor seals and adult female and/or juvenile harbor seals as measured by crude protein, lipid, ash, and GE did not differ significantly (Wilcoxon signed rank test, $p > 0.05$; Figure 2.7).

DISCUSSION

Analysis of corticosteroid metabolite profiles from free-ranging harbor seal scats yielded two distinct patterns of metabolite profiles that matched those from a known adult male and known adult females and/or a juvenile male. Previous observations of juveniles in other species, such as Steller sea lions, have noted a lack of sex-specific differences in corticosteroid metabolite profiles that are seen in adults (Mashburn & Atkinson 2008). In this study, we did not have any samples available from a known juvenile female harbor seal, so sex specific differences between juvenile harbor seals could not be evaluated. However, the corticosteroid metabolite profiles obtained from the adult females and the juvenile male were indistinguishable.

The metabolism of glucocorticoids, like corticosterone, is complex and the dominant metabolites (Figure 2.1) identified in feces differ depending on species, sex (Wasser et al. 2000, Mashburn & Atkinson 2004, Touma & Palme 2005, Keay et al. 2006, Mashburn & Atkinson 2007), and age class (Mashburn & Atkinson 2008) of the animal. The variation in corticosteroid metabolism and the resulting metabolites in feces can arise from a number of sources, including different enzymatic pathways resulting from differential gene expression and interaction with other hormones circulating through the body (von der Ohe & Servheen 2002, Goymann 2005). However, the exact cause for variation is usually unclear (Touma et al. 2003). Consequently, there is potential for variation depending on the individual. While we were able to observe a consistent metabolite pattern for all three known adult females, only one adult male and one

juvenile male harbor seal were sampled. Future studies should include additional known sex and age samples to evaluate and account for potential individual variation.

Other hormones also show sex-specific differences. Major reproductive steroid hormones, such as estrogen, progesterone, and testosterone, can show sex-specific differences in both the metabolite profiles as well as the overall concentration of hormones in the feces (Oates et al. 2002, Goymann 2005, Rolland et al. 2005, Szymanski et al. 2006). However, analysis of sex steroid hormone concentration in scats may be confounded by a variety of factors. The concentration of both reproductive hormones and glucocorticoids may vary not only between the sexes of the animals, but also by a host of other factors including the time of day, the season, stress levels, reproductive status, and social status (Atkinson 1997, Goymann et al. 2001, Huber et al. 2003, Touma et al. 2003, Palme et al. 2005, Rolland et al. 2005, Landys et al. 2006, Greig et al. 2007, Mashburn & Atkinson 2008, Pomeroy 2011). However, previous studies have demonstrated distinct differences in male and female corticosteroid metabolite profiles in several species including Steller sea lion (Touma et al. 2003, Mashburn & Atkinson 2004, Rettenbacher et al. 2004, Baltic et al. 2005). Therefore, although differences in profiles of other hormones may exist, for this study we only considered metabolite profiles of corticosteroids.

Pinnipeds of different sexes and age classes may consume different diets depending on a variety of factors including foraging strategies resulting from sexual segregation, behavior during the breeding and molting seasons, and diving capabilities (Thompson et al. 1989, Le Boeuf et al. 2000, Page et al. 2005, Beck et al. 2007). Northern elephant seals (*Mirounga angustirostris*) exhibit sexual segregation as males will forage on potentially more energy-dense prey located along the coast of North America, at a higher risk of predation by orcas (*Orcinus orca*) and white sharks (*Carcharodon carcharius*), to amass the body size and condition necessary to compete with other males for access to females (Le Boeuf et al. 2000). Female northern elephant seals are

smaller and tend to forage in the potentially less hazardous open ocean with reduced risk of predation, on more diffusely spread prey items, because their reproductive fitness does not depend on size but on successful rearing of pups (Le Boeuf et al. 2000). Sex- or age-specific diving capabilities also may affect the prey species that a harbor seal is able to access. Gray seals (*Halichoerus grypus*) in Nova Scotia, Canada, exhibit diet differences between the sexes, which may result from different energetic needs as part of breeding season behaviors (Beck et al. 2007). Australian and New Zealand fur seals (*Arctocephalus pusillus doriferus* and *A. forsteri*, respectively) also demonstrate diet differences depending on sex and age class of the animal (Page et al. 2005). Adult males of both species may be better able to capture and handle larger prey items, so these animals tend to consume larger, more energy-rich prey species than do smaller adult females and juveniles with different diving capabilities (Page et al. 2005).

Even though harbor seals are not markedly sexually dimorphic and do not exhibit different diving behaviors between sexes and age classes (Hastings et al. 2004), sex- or age-specific behavioral patterns have been observed during the breeding and molting periods (Thompson et al. 1989, Coltman et al. 1997) that may lead to diet differentiation between these groups. Due to behavioral differences between male and female harbor seals, such as time devoted to foraging and foraging range, the sex and age classes may have access to different prey species of varying nutritional quality. Thus, changes to these different prey bases could impact one sex or age group more significantly than another. Therefore, it is important to consider seal age and sex when evaluating diet.

The samples for this study were collected during the molting period, which on Tugidak Island runs from late July through September (Daniel et al. 2003). During the molting period, harbor seals will haul out more frequently than during other times of the year (Thompson et al. 1989). This is likely because warmer skin temperatures and increased blood perfusion to the

epidermis stimulates follicles and hair growth (Feltz & Fay 1966). Immersion in cold water makes thermal homeostasis too energetically costly to maintain due to increased blood perfusion to the skin rather than the usual peripheral vasoconstriction (Feltz & Fay 1966, Boily 1995). While adult males and juveniles tend to reduce their foraging trips, potentially to remain on land longer to speed up the progression of their molt, post-lactation adult females may make more extensive foraging trips (Thompson et al. 1989). These females have just spent most of their energy reserves on providing energy rich milk for their pup and the need to restore their fat reserves may outweigh the costs associated with a slower progression of their molt resulting from spending more time in the water (Thompson et al. 1989). Therefore, there was potential for adult females to have foraged on different prey species than other harbor seals during this time period.

Diets consumed by adult males and adult females/juveniles were inferred by matching metabolite profiles with the prey items identified in the scats that the hormone samples came from. The SDI of both the presumed adult male and the adult female/juvenile harbor seal groups was low (<2.1; maximum SDI=20), but the SDI of adult females/juvenile group was found to be significantly higher than that of the adult males. The presumed adult females/juveniles were found to consume three prey species that were not observed in the diets of adult males (capelin, salmon species, and sand lance) and this may have contributed to the observed difference in diet diversity. Higher dependence on a few prey items or uneven consumption of prey species can contribute to lower SDI. Two prey species could have accounted for approximately 50% of the average diet of the male harbor seals, greenling species and rock sole, whereas for adult females/juveniles, at least three prey species were required to account for 50% of the average diet. This supports the hypothesis that adult females may have more diverse diets during the molting period because these females likely partook in longer foraging trips during this time period.

It is possible that the difference in diet diversity between sexes may have been an effect of small sample size. Prior studies have estimated the minimum number of scats needed to detect a difference between groups over time, between regions, or between sex or age groups (Hammond & Rothery 1996, Trites et al. 2005). These studies estimated that a minimum of 59 scats is recommended to accurately identify principle prey items occurring in >5% of scats (Trites et al. 2005), and a minimum of 94 (Trites et al. 2005) to 100 (Hammond & Rothery 1996) scat samples from each group should be used to determine at least moderate differences. Therefore, the results of this study should be viewed cautiously because our sample sizes were not sufficiently large (adult males: $n = 6$; adult females or juveniles: $n = 10$) to confidently detect a difference between groups. However, the diet diversity herein was consistent with that reported in Chapter 1, where a larger sample size was available.

Small sample sizes in the present study may have impacted the average relative importance of certain prey species. For example, salmon species only occurred in two of the ten adult female/juvenile harbor seal samples. However, when salmon was observed, it comprised about 99% of the estimated consumed biomass leading to an approximate relative importance of salmon in the average diet of about 20%. Irish lord species were observed in five of the ten samples and contributed variably to the estimated consumed biomass (range: 10.3-87.9%), but was also found to comprise about 20% of the average diet for all samples in the adult female/juvenile harbor seal group. Therefore, Irish lord species, which appeared to be consumed more frequently in more variable quantities, would contribute the same as salmon species, which were consumed less frequently, but in very large quantities. Inclusion of more samples may help identify if these species were in fact equivalent in average relative importance or if this was an artifact of small sample sizes.

The salmon consumption observed in the present study may have been influenced by biases associated with estimation of consumed BR. Because BR assumes that the predator consumes the entire prey item, there was potential for overestimation of consumed biomass, especially for large prey items like salmon, which may not have been entirely ingested (Wright et al. 2007, Hauser et al. 2008, Phillips & Harvey 2009). Harbor seals have been observed selectively eating specific parts of salmon, namely the energy-rich abdomen and eggs of female salmon, and abandoning the rest of the carcass (Hauser et al. 2008). Because we could not account for the amount of prey actually consumed compared to the portion that was discarded, we assumed that the entire estimated biomass was ingested. Partial consumption of salmon complicates analysis not only by introducing the possibility of overestimating relative importance, but by also presenting potential for underestimating importance. If harbor seals specifically targeted bellies and eggs of female salmon then they were also consuming higher energy density material, which then could have impacted our nutritional analysis (Gende et al. 2004, Hauser et al. 2008). Many prey species are expected to exhibit seasonality in proximate composition and nutritional quality, but availability of these data was limited. In this study, the MIXIT nutritional analysis estimated that the diets consumed by adult male and adult female/juvenile harbor seals was not significantly different in terms of nutritional quality. However, the partial consumption of energy rich salmon eggs by adult female/juvenile harbor seals could not be determined and this could have impacted our results. The MIXIT program is only as useful as the data used to construct the prey database, so additional research into seasonality in prey proximate composition in different regions should be encouraged.

Prey DNA analysis could help to identify the presence of salmon in the absence of identifiable hard-parts (Parsons et al. 2005, Tollit et al. 2006, Casper et al. 2007); however, genetic information for all prey species is required, estimates of amount of prey consumed cannot

be obtained from DNA yet, and fecal DNA quantity and quality can also be problematic (Reed et al. 1997, Taberlet et al. 1999, Tollit et al. 2006, Broquet et al. 2007, Deagle & Tollit 2007). One area where DNA analysis may be especially useful is in increasing species-specific resolution of identified hard parts (e.g., identifying salmon to species) to improve dietary analysis (Parsons et al. 2005, Tollit et al. 2009). There has also been growing interest in the use of DNA to identify the individual animal that produced the scat, but there are several challenges with employing these techniques effectively. Limitations involved with fecal DNA analysis include low DNA concentrations, DNA degradation in the sample, and risk of contamination by foreign DNA present in the sample (Reed et al. 1997, Taberlet et al. 1999, Broquet et al. 2007). DNA analysis also does not allow for the possibility of distinguishing age classes, which can be an important consideration for diet studies. Combination of corticosteroid metabolite profiles with other types of analysis (such as DNA analysis) could help separate adult females and juvenile males from each other (i.e., use DNA to ID sex within that group to separate juvenile males from females). Future studies should explore this methodology to assist in dietary studies as well as sex determination of free-ranging marine mammal scats.

This study utilized a new method to identify harbor seal sex and age classes from scats obtained from unknown animals. Adult harbor seal males produced different fecal corticosteroid metabolite profiles than adult females/juveniles, and these profiles were used to determine the sex and age class of unknown harbor seals from scat samples. Subsequently, these methods can be used to identify differences in the diets consumed by sex and age classes. Dietary differences between the sex and age classes can have important implications for the management of harbor seals and their prey around Tugidak Island. In this study, the diet diversity of the presumed adult males was significantly lower than that of the presumed adult females and/or juveniles. However, the nutritional quality of the diets of these groups was not significantly different. As generalist

predators, harbor seals readily take advantage of available prey and even when diet diversity was reduced, these animals appeared to be able to maintain their nutritional input. Future studies should evaluate additional known sex and age harbor seal metabolite profiles collected throughout the year to account for potential individual and seasonal variation.

FIGURES

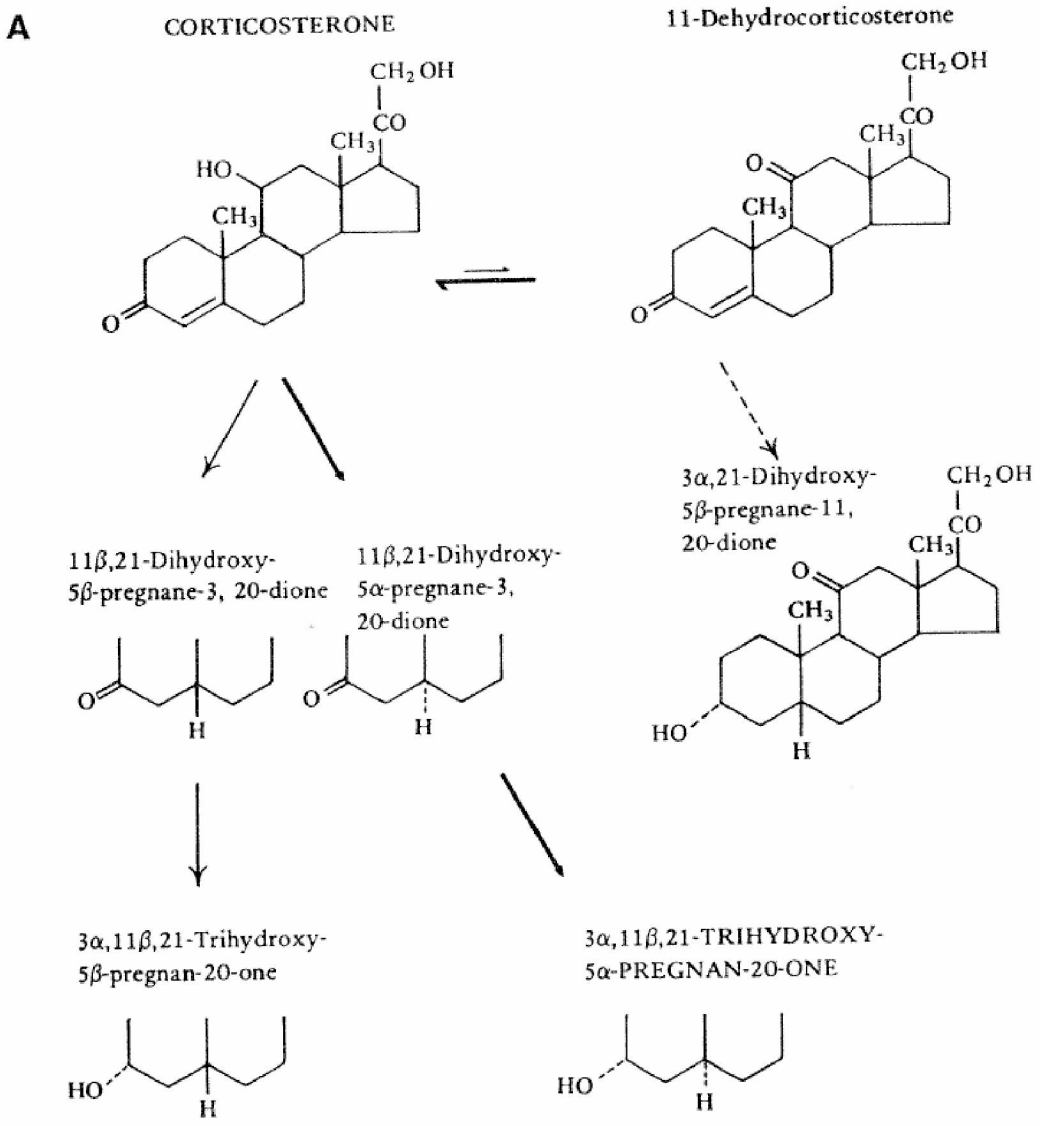

Figure 2.1: Structure of corticosterone and various metabolites (Norman & Litwack 1997). These metabolites might be present in sample fractions eluted through the HPLC.

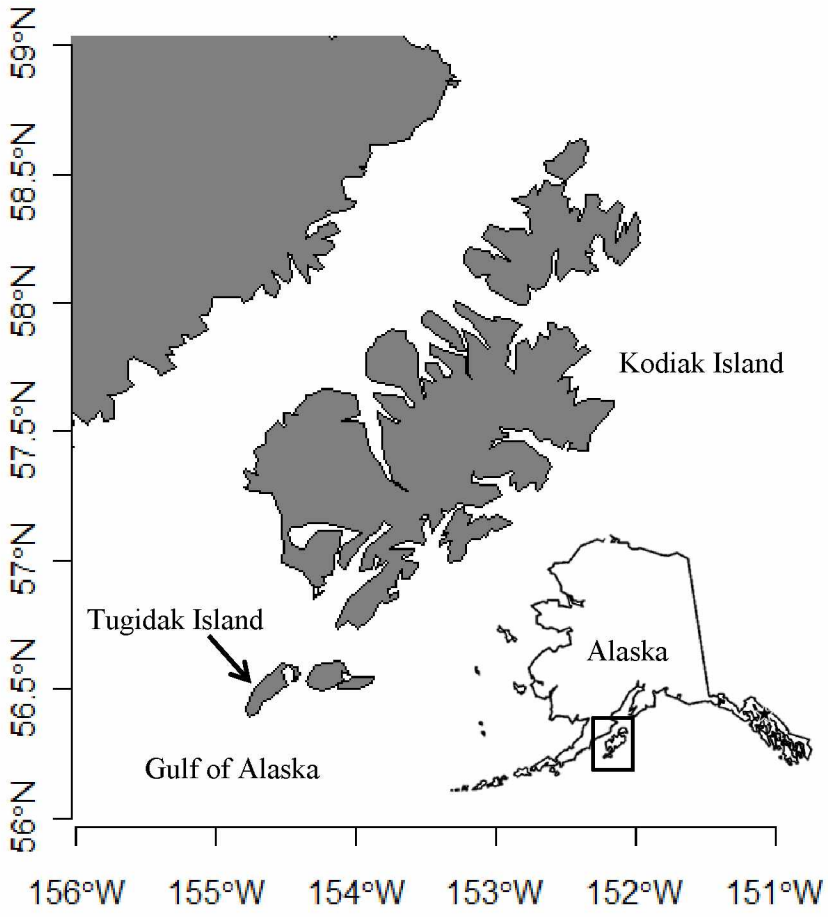

Figure 2.2: Location of Tugidak Island in the western Gulf of Alaska.

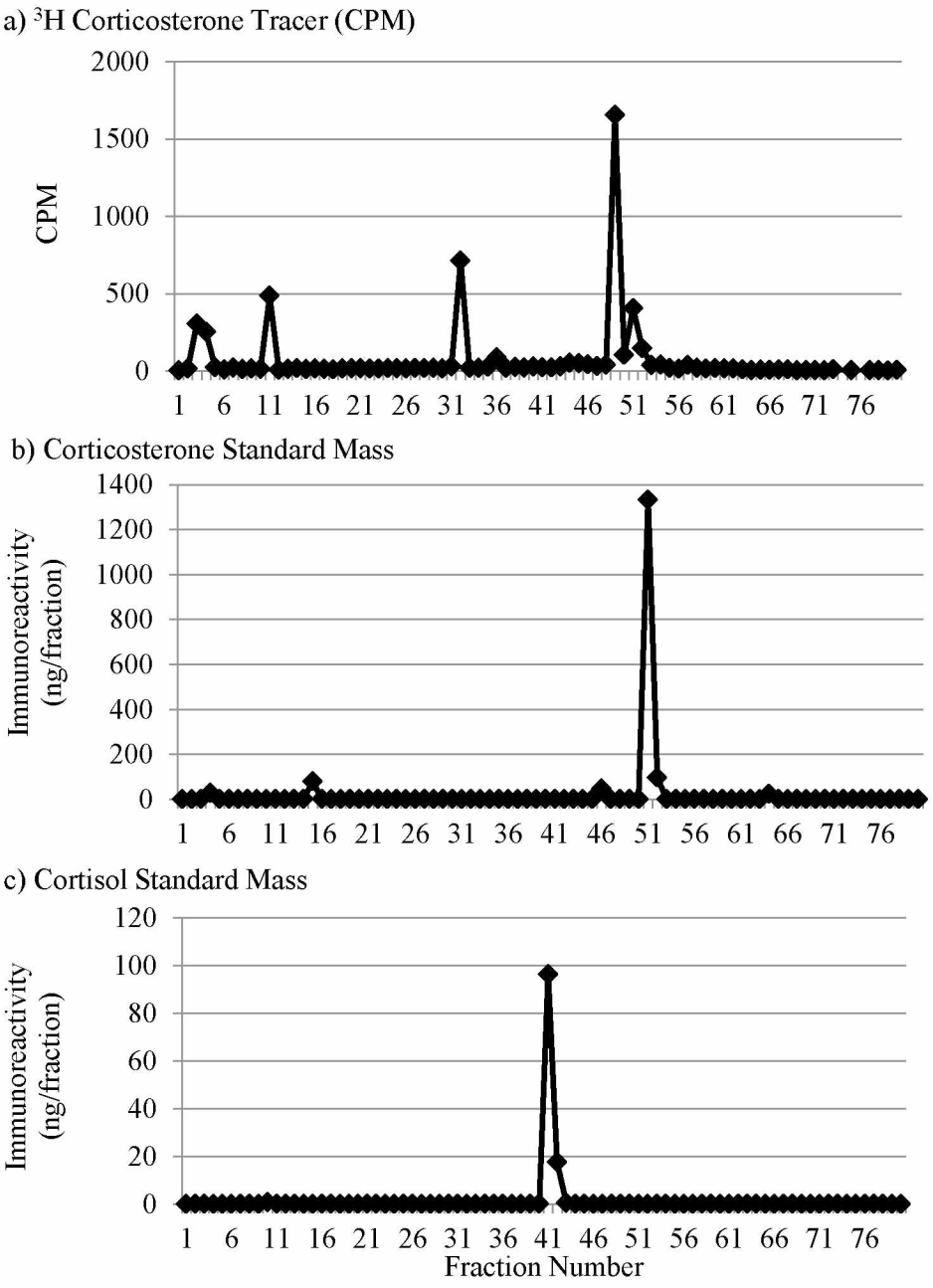

Figure 2.3: HPLC standard runs. a) ^{3}H-corticosterone tracer in 100% methanol (peak at fraction 49-51); b) Corticosterone standard (peak at fraction 49-51); c) Cortisol standard (peak at fraction 41-42).

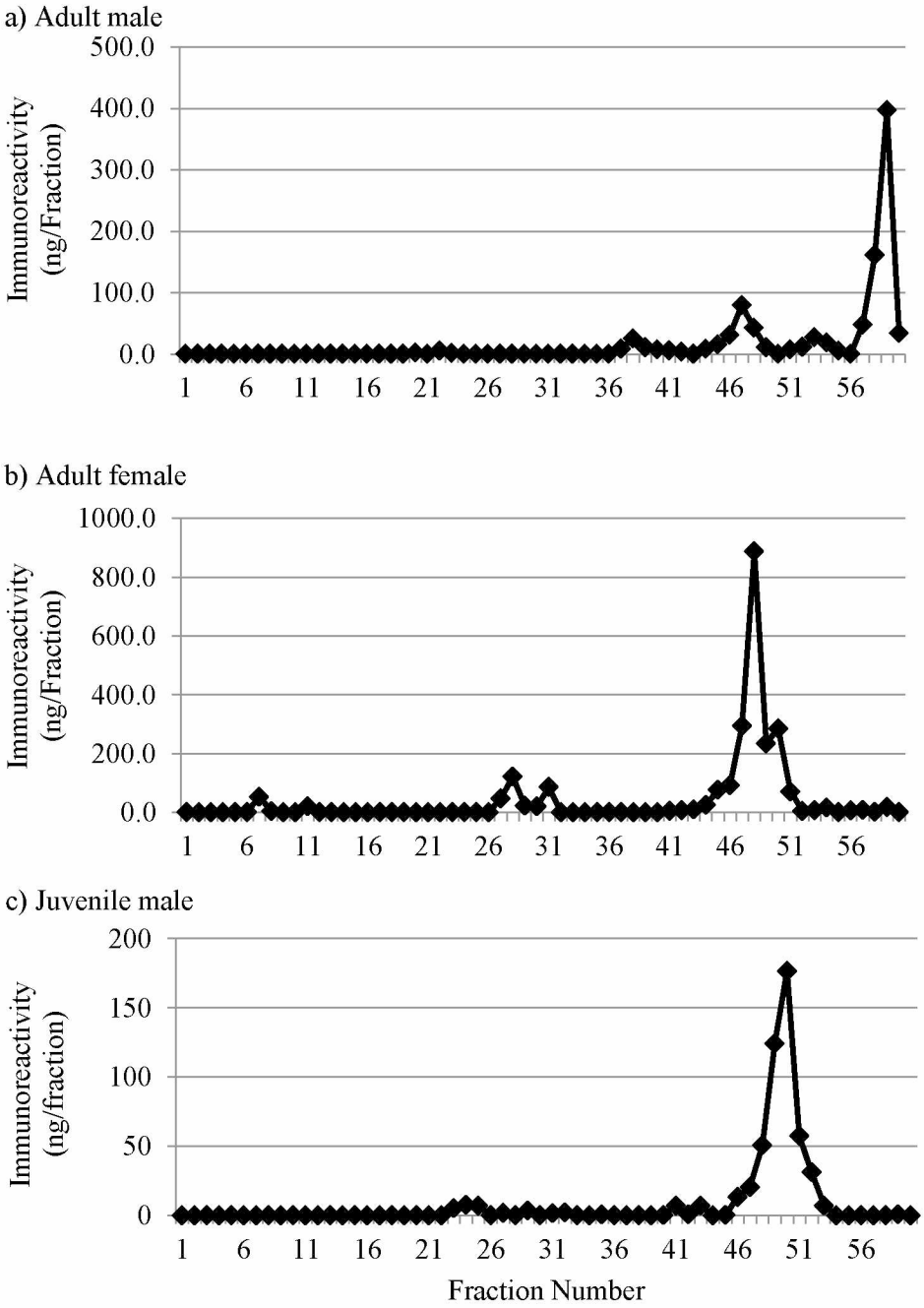

Figure 2.4: Corticosteroid immunoreactivity peaks for captive known-sex harbor seals (*Phoca vitulina*). a) Adult male showed immunoreactive peaks between fractions 46-49 and between fractions 55-60; b) Representative profile of one of the adult females, all females showed an immunoreactive peak between fractions 46-52; c) The profile of the juvenile male showed only one immunoreactive peak between fractions 47-52, similar to the adult female profile.

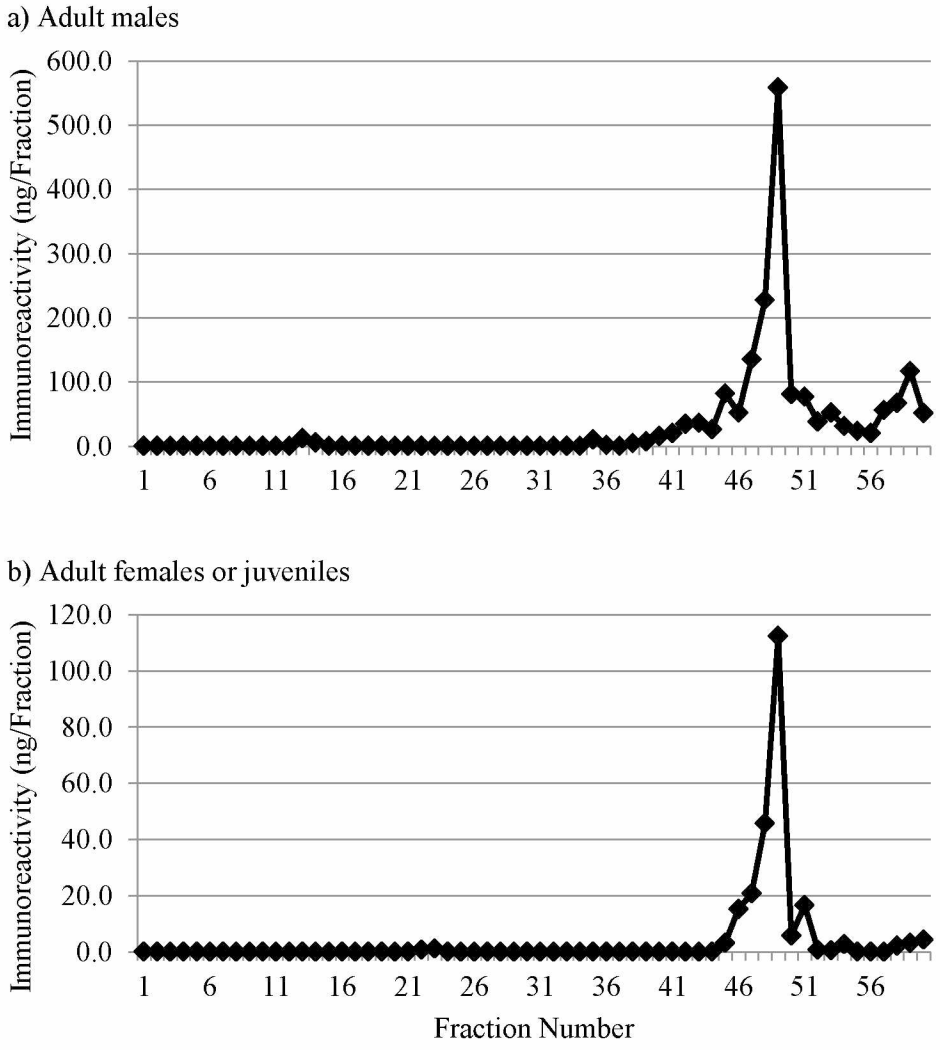

Figure 2.5: Radioimmunoassay profiles of fecal corticosteroid metabolites. Scats collected from harbor seal (*Phoca vitulina*) scats collected from Tugidak Island, Alaska. Immunoreactivity from fractions of high-performance liquid chromatography portrayed two immunoreactivity patterns: a) Adult males (n=6): immunoreactive peaks between fractions 46-52 with a second peak between fractions 55-60; b) Adult females or juveniles (n=10): dominant peaks between fractions 46-52 with no second peak.

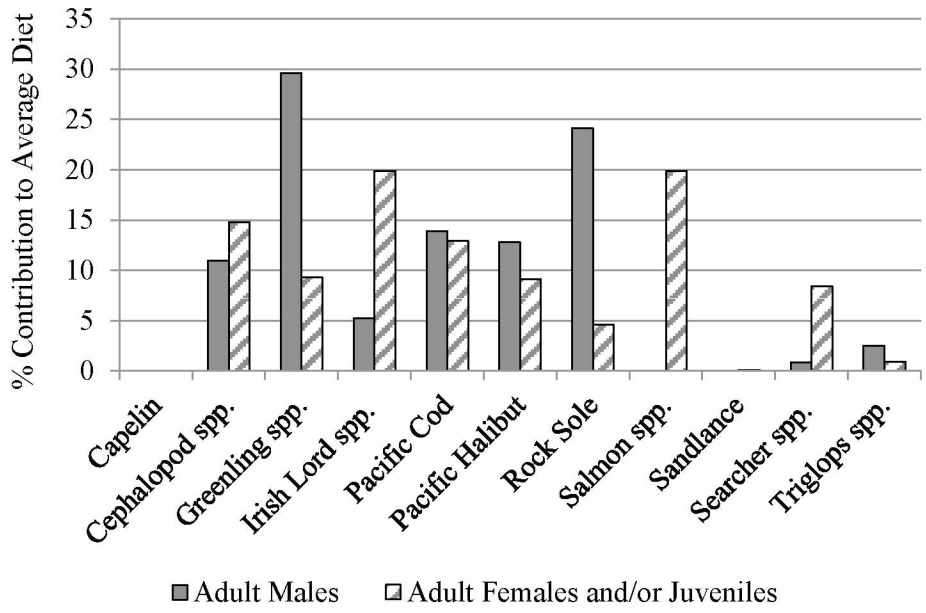

Figure 2.6: Relative importance of prey species between free-ranging harbor seal (*Phoca vitulina*) groups. Adult male (n=6) and adult female and/or juvenile (n=10) groups determined by fecal corticosteroid immunoreactivity profiles. Relative importance is calculated by percent contribution to total estimated biomass consumed.

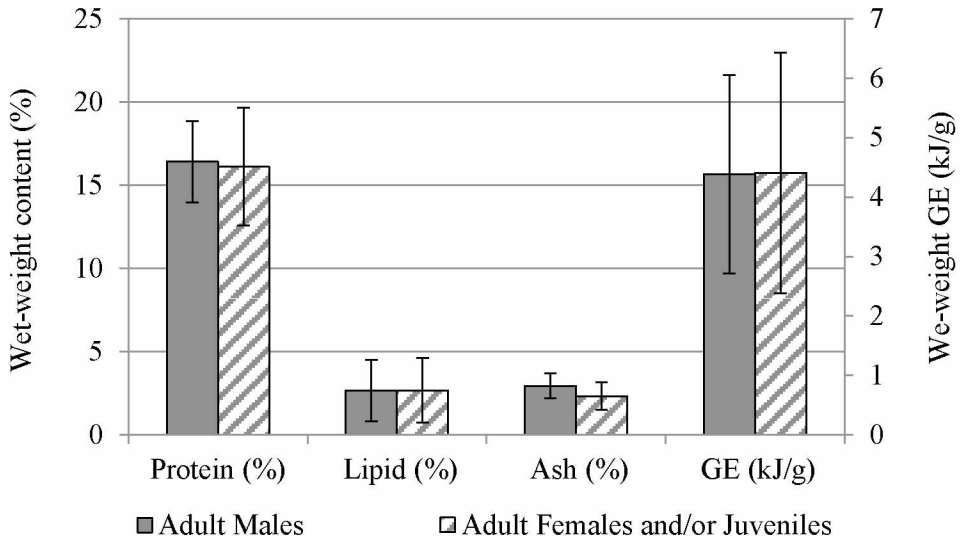

Figure 2.7: Average nutritional composition of harbor seal (*Phoca vitulina*) diets. Nutritional composition of diets from presumed adult males (n=6) and adult females and/or juveniles (n=10) identified by fecal corticosteroid immunoreactivity profiles calculated using the MIXIT-WIN computer program (Agricultural Software Consultants Inc., San Diego, California). All nutrient parameters are given on a wet-weight basis. Error bars represent standard deviations. Nutritional parameters are not significantly different between adult male and adult female and/or juvenile harbor seals (Wilcoxon signed rank test, p>0.05).

LITERATURE CITED

Alverson DL (1991) Commercial fisheries and the Steller sea lion (*Eumetopias jubatus*): The conflict arena. Fisheries Research Institute, School of Fisheries, University of Washington, Seattle, WA, p99

Andersen SM, Lydersen C, Grahl-Nielsen O, Kovacs KM (2004) Autumn diet of harbour seals (*Phoca vitulina*) at Prins Karls Forland, Svalbard, assessed via scat and fatty-acid analyses. Canadian Journal of Zoology 82:1230-1245

Atkinson S (1997) Reproductive biology of seals. Reviews of Reproduction 2:175-194

Baltic M, Jenni-Eiermann S, Arlettaz R, Palme R (2005) A noninvasive technique to evaluate human-generated stress in the black grouse. Annals of the New York Academy of Sciences 1046:81-95

Beck CA, Iverson SJ, Bowen WD, Blanchard W (2007) Sex differences in grey seal diet reflect seasonal variation in foraging behaviour and reproductive expenditure: evidence from quantitative fatty acid signature analysis. Journal of Animal Ecology 76:490-502

Berg I, Haug T, Nilssen KT (2002) Harbour seal (*Phoca vitulina*) diet in Vesterålen, North Norway. Sarsia - North Atlantic Marine Science 87:451-461

Bleich VC, Bowyer RT, Wehausen JD (1997) Sexual segregation in mountain sheep: resources or predation? Wildlife Monographs 134:3-50

Boily P (1995) Theoretical heat flux in water and habitat selection of phocid seals and beluga whales during the annual molt. Journal of Theoretical Biology 172:235-244

Boness D, Bowen W, Oftedall O (1994) Evidence of a maternal foraging cycle resembling that of otariid seals in a small phocid, the harbor seal. Behavioral Ecology and Sociobiology 34:95-104

Boness DJ, Bowen WD (1996) The evolution of maternal care in pinnipeds. Bioscience 46:645-654

Boness DJ, Bowen WD, Buhleier BM, Marshall GJ (2006) Mating tactics and mating system of an aquatic-mating pinniped: the harbor seal, *Phoca vitulina*. Behavioral Ecology and Sociobiology 61:119-130

Bowen WD, Oftedal OT, Boness DJ (1992) Mass and energy transfer during lactation in a small phocid, the harbor seal (*Phoca vitulina*). Physiological Zoology 65:844-866

Bowen W, Boness D, Iverson S (1999) Diving behaviour of lactating harbour seals and their pups during maternal foraging trips. Canadian Journal of Zoology 77:978-988

Broquet T, Ménard N, Petit E (2007) Noninvasive population genetics: a review of sample source, diet, fragment length and microsatellite motif effects on amplification success and genotyping error rates. Conservation Genetics 8:249-260

Burns JJ (2009) Harbor seal and spotted seal. In: Perrin WF, Würsig B, Thewissen JGM (eds) Encyclopedia of Marine Mammals. Academic Press, San Francisco

Casper R, Gales N, Hindell M, Robinson S (2006) Diet estimation based on an integrated mixed prey feeding experiment using *Arctocephalus* seals. Journal of Experimental Marine Biology and Ecology 328:228-239

Casper RM, Jarman SN, Gales NJ, Hindell MA (2007) Combining DNA and morphological analyses of faecal samples improves insight into trophic interactions: a case study using a generalist predator. Marine Biology 152:815-825

Clarke MR (1962) The identification of cephalopod" beaks" and the relationship between beak size and total body weight. Bulletin of the British Museum (Natural History) Zoology 8 (10):419-480

Clarke MR (1986) A handbook for the identification of cephalopod beaks. Oxford University Press, New York,

Coltman DW, Bowen W, Boness DJ, Iverson SJ (1997) Balancing foraging and reproduction in the male harbour seal, an aquatically mating pinniped. Animal Behaviour 54:663-678

Conners ME, Conrath C (2010) Assessment of the octopus complex in the Gulf of Alaska. Alaska Fisheries Science Center, North Pacific Fisheries Management Council Gulf of Alaska SAFE Report, p745-776

Cottrell P, Trites A, Miller E (1996) Assessing the use of hard parts in faeces to identify harbour seal prey: results of captive-feeding trials. Canadian Journal of Zoology 74:875-880

Crowley PH (1992) Resampling methods for computation-intensive data analysis in ecology and evolution. Annual Review of Ecology and Systematics 23:405-447

Daniel RG, Jemison LA, Pendleton GW, Crowley SM (2003) Molting phenology of harbor seals on Tugidak Island, Alaska. Marine Mammal Science 19:128-140

Deagle B, Tollit D (2007) Quantitative analysis of prey DNA in pinniped faeces: potential to estimate diet composition? Conservation Genetics 8:743-747

Feltz ET, Fay FH (1966) Thermal requirements in vitro of epidermal cells from seals. Cryobiology 3:261-264

Gende SM, Quinn TP, Willson MF, Heintz R, Scott TM (2004) Magnitude and fate of salmon-derived nutrients and energy in a coastal stream ecosystem. Journal of Freshwater Ecology 19:149-160

Goymann W, East ML, Wachter B, Höner OP, Möstl E, Van't Holf TJ, Hofer H (2001) Social, state-dependent and environmental modulation of faecal corticosteroid levels in free-ranging female spotted hyenas. Proceedings of the Royal Society of London Series B: Biological Sciences 268:2453-2459

Goymann W, Möstl E, Gwinner E, Karasov W (2002) Corticosterone metabolites can be measured noninvasively in excreta of European stonechats (*Saxicola torquata rubicola*). The Auk 119:1167-1173

Goymann W (2005) Noninvasive monitoring of hormones in bird droppings: physiological validation, sampling, extraction, sex differences, and the influence of diet on hormone metabolite levels. Annals of the New York Academy of Sciences 1046:35-53

Greig DJ, Mashburn KL, Rutishauser M, Gulland FMD, Williams TM, Atkinson S (2007) Seasonal changes in circulating progesterone and estrogen concentrations in the California sea lion (*Zalophus californianus*). Journal of Mammalogy 88:67-72

Hammond P, Rothery P (1996) Application of computer sampling in the estimation of seal diet. Journal of Applied Statistics 23:525-534

Hastings K, Frost K, Simpkins M, Pendleton G, Swain U, Small R (2004) Regional differences in diving behavior of harbor seals in the Gulf of Alaska. Canadian Journal of Zoology 82:1755-1773

Hauser DDW, Allen CS, Rich HB, Quinn TP (2008) Resident harbor seals (*Phoca vitulina*) in Iliamna Lake, Alaska: summer diet and partial consumption of adult sockeye salmon (*Oncorhynchus nerka*). Aquatic Mammals 34:303-309

Herreman JK, Blundell GM, Ben-David M (2009) Evidence of bottom-up control of diet driven by top-down processes in a declining harbor seal *Phoca vitulina richardsi* population. Marine Ecology Progress Series 374:287-300

Huber HR, Jorgensen JC, Butler VL, Baker G, Stevens R (2011) Can salmonids (*Oncorhynchus* spp.) be identified to species using vertebral morphometrics? Journal of Archaeological Science 38:136-146

Huber S, Palme R, Arnold W (2003) Effects of season, sex, and sample collection on concentrations of fecal cortisol metabolites in red deer (*Cervus elaphus*). General and Comparative Endocrinology 130:48-54

Jemison LA, Kelly BP (2001) Pupping phenology and demography of harbor seals (*Phoca vitulina richardii*) on Tugidak Island, Alaska. Marine Mammal Science 17:585-600

Jemison L, Pendleton G, Wilson C, Small R (2006) Long-term trends in harbor seal numbers at Tugidak Island and Nanvak Bay, Alaska. Marine Mammal Science 22:339-360

Kacsoh B (2000) Endocrine physiology. McGraw-Hill Medical, New York, NY, p392-429

Keay JM, Singh J, Gaunt MC, Kaur T (2006) Fecal glucocorticoids and their metabolites as indicators of stress in various mammalian species: a literature review. Journal of Zoo and Wildlife Medicine 37:234-244

Kirsch PE, Iverson SJ, Bowen WD (2000) Effect of a low-fat diet on body composition and blubber fatty acids of captive juvenile harp seals (*Phoca groenlandica*). Physiological and Biochemical Zoology 73:45-59

Landys MM, Ramenofsky M, Wingfield JC (2006) Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. General and Comparative Endocrinology 148:132-149

Le Boeuf B, Crocker D, Costa D, Blackwell S, Webb P, Houser D (2000) Foraging ecology of northern elephant seals. Ecological Monographs 70:353-382

Mashburn KL, Atkinson S (2004) Evaluation of adrenal function in serum and feces of Steller sea lions (*Eumetopias jubatus*): influences of molt, gender, sample storage, and age on glucocorticoid metabolism. General and Comparative Endocrinology 136:371-381

Mashburn KL, Atkinson S (2007) Seasonal and predator influences on adrenal function in adult Steller sea lions: Gender matters. General and Comparative Endocrinology 150:246-252

Mashburn KL, Atkinson S (2008) Variability in leptin and adrenal response in juvenile Steller sea lions (*Eumetopias jubatus*) to adrenocorticotropic hormone (ACTH) in different seasons. General and Comparative Endocrinology 155:352-358

Mathisen OA, Lopp RJ (1963) Photographic census of the Steller sea lion herds in Alaska, 1956-1958. United States Fish and Wildlife Service, Special Scientific Report of Fisheries 424

McKenzie J, Wynne KM (2008) Spatial and temporal variation in the diet of Steller sea lions in the Kodiak Archipelago, 1999 to 2005. Marine Ecology Progress Series 360:265-283

Merrick RL, Chumbley MK, Byrd GV (1997) Diet diversity of Steller sea lions (*Eumetopias jubatus*) and their population decline in Alaska: a potential relationship. Canadian Journal of Fisheries and Aquatic Sciences 54:1342-1348

Middlemas SJ, Barton TR, Armstrong JD, Thompson PM (2006) Functional and aggregative responses of harbour seals to changes in salmonid abundance. Proceedings: Biological Sciences 273:193-198

Monfort SL, Mashburn K, Brewer B, Creel S (1998) Evaluating adrenal activity in African wild dogs (*Lycaon pictus*) by fecal corticosteroid analysis. Journal of Zoo and Wildlife Medicine 29:129-133

Möstl E, Palme R (2002) Hormones as indicators of stress. Domestic Animal Endocrinology 23:67-74

Möstl E, Rettenbacher S, Palme R (2005) Measurement of corticosterone metabolites in birds' droppings: an analytical approach. Annals of the New York Academy of Sciences 1046:17-34

Nelson R (2000) An introduction to behavioral endocrinology. Sinauer Associeates, Inc., Sunderland, MA, p78-591

Norman AW, Litwack G (1997) Hormones. Academic Press, San Deigo, CA, p281-310

Norris DO (1997) Vertebrate endocrinology. Academic Press, San Diego, CA, p305-310

Oates J, Bradshaw F, Bradshaw S, Lonsdale R (2002) Sex identification and evidence of gonadal activity in the short-beaked echidna (*Tachyglossus aculeatus*) (Monotremata: Tachyglossidae): non-invasive analysis of faecal sex steroids. Australian Journal of Zoology 50:395-406

Oki C, Atkinson S (2004) Diurnal patterns of cortisol and thyroid hormones in the harbor seal (*Phoca vitulina*) during summer and winter seasons. General and Comparative Endocrinology 136:289-297

Orchard TJ (2003) An application of the linear regression technique for determining length and weight of six fish taxa: the role of selected fish species in Aleut paleodiet. Archaeopress Oxford, United Kingdom

Page B, McKenzie J, Goldsworthy SD (2005) Dietary resource partitioning among sympatric New Zealand and Australian fur seals. Marine Ecology Progress Series 293:283-302

Palme R, Rettenbacher S, Touma C, El-Bahr S, Möstl E (2005) Stress hormones in mammals and birds: comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. Annals of the New York Academy of Sciences 1040:162-171

Parsons KM, Piertney SB, Middlemas SJ, Hammond PS, Armstrong JD (2005) DNA-based identification of salmonid prey species in seal faeces. Journal of Zoology 266:275-281

Phillips EM, Harvey JT (2009) A captive feeding study with the Pacific harbor seal (*Phoca vitulina richardii*): Implications for scat analysis. Marine Mammal Science 25:373-391

Pitcher K (1980) Stomach contents and feces as indicators of harbor seal, *Phoca vitulina*, foods in the Gulf of Alaska. Fishery Bulletin 78:797-798

Pitcher KW (1990) Major decline in number of harbor seals, *Phoca vitulina richardsi*, on Tugidak Island, Gulf of Alaska. Marine Mammal Science 6:121-134

Pomeroy P (2011) Reproductive cycles of marine mammals. Animal Reproduction Science 124:184-193

Reed J, Tollit D, Thompson P, Amos W (1997) Molecular scatology: the use of molecular genetic analysis to assign species, sex and individual identity to seal faeces. Molecular Ecology 6:225-234

Reeves RR, Stewart BS, Clapham PJ, Powell JA (2002) Guide to marine mammals of the world. AA Knopf Inc., New York, p118-121

Rettenbacher S, Möstl E, Hackl R, Ghareeb K (2004) Measurement of corticosterone metabolites in chicken droppings. British Poultry Science 45:704-711

Robinson SMC, Hartwick EB (1983) The relationship between beak morphometrics and the live wet weight of the giant Pacific octopus, *Octopus dofleini martini* (Wülker). The Veliger 26:26-29

Rolland RM, Hunt KE, Kraus SD, Wasser SK (2005) Assessing reproductive status of right whales (*Eubalaena glacialis*) using fecal hormone metabolites. General and Comparative Endocrinology 142:308-317

Rosen D, Trites A (2000) Pollock and the decline of Steller sea lions: testing the junk-food hypothesis. Canadian Journal of Zoology 78:1243-1250

Schwarzenberger F (2007) The many uses of non-invasive faecal steroid monitoring in zoo and wildlife species. International Zoo Yearbook 41:52-74

Shannon CEW (1949) The mathematical theory of communication. Urbana, Univ of Illinois Press

Sigler M, Tollit D, Vollenweider J, Thedinga J, Csepp D, Womble J, Wong M, Rehberg M, Trites A (2009) Steller sea lion foraging response to seasonal changes in prey availability. Marine Ecology Progress Series 388:243-261

Sinclair E, Zeppelin T (2002) Seasonal and spatial differences in diet in the western stock of Steller sea lions (*Eumetopias jubatus*). Alaska Fisheries Science Center, National Marine Fisheries Service, NOAA, p1-42

Small R, Pendleton G, Pitcher K (2003) Trends in abundance of Alaska harbor seals, 1983-2001. Marine Mammal Science 19:344-362

Southward GM, Hardman WH (1973) Otolith length and fish length of Pacific halibut. International Pacific Halibut Commission, p10

Stanberry K (2003) The effect of changes in dietary fat level on body composition, blood metabolites and hormones, rate of passage, and nutrient assimilation efficiency in harbor seals. Master of Science, University of Hawaii at Manoa, Honolulu, HI, p116

Szymanski D, Gist D, Roth T (2006) Anuran gender identification by fecal steroid analysis. Zoo Biology 25:35-46

Taberlet P, Waits LP, Luikart G (1999) Noninvasive genetic sampling: look before you leap. Trends in Ecology and Evolution 14:323-327

Thompson P, Fedak M, McConnell B, Nicholas K (1989) Seasonal and sex-related variation in the activity patterns of common seals (*Phoca vitulina*). Journal of Applied Ecology 26:521-535

Thompson PM, Miller D, Cooper R, Hammond PS (1994) Changes in the distribution and activity of female harbour seals during the breeding season: implications for their lactation strategy and mating patterns. Journal of Animal Ecology 63:24-30

Tollit D, Black A, Thompson P, Mackay A, Corpe H, Wilson B, Parijs S, Grellier K, Parlane S (1998) Variations in harbour seal *Phoca vitulina* diet and dive-depths in relation to foraging habitat. Journal of Zoology 244:209-222

Tollit D, Heaslip S, Deagle B, Iverson S, Joy R, Rosen D, Trites A (2006) Estimating diet composition in sea lions: which technique to choose? In: Trites A, Atkinson S, DeMaster D, Fritz L, Gelatt T, Rea L, Wynne K (eds) Sea lions of the world. Alaska Sea Grant College Program, University of Alaska, Fairbanks, AK, p293–308

Tollit D, Schulze A, Trites A, Olesiuk P, Crockford S, Gelatt T, Ream R, Miller K (2009) Development and application of DNA techniques for validating and improving pinniped diet estimates. Ecological Applications 19:889-905

Touma C, Sachser N, Möstl E, Palme R (2003) Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. General and Comparative Endocrinology 130:267-278

Touma C, Palme R (2005) Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. Annals of the New York Academy of Sciences 1046:54-74

Trites AW, Donnelly CP (2003) The decline of Steller sea lions *Eumetopias jubatus* in Alaska: a review of the nutritional stress hypothesis. Mammal Review 33:3-28

Trites AW, Joy R, Weckerly FW (2005) Dietary analysis from fecal samples: How many scats are enough? Journal of Mammalogy 86:704-712

Trites AW, Calkins DG, Winship AJ (2007) Diets of Steller sea lions (*Eumetopias jubatus*) in Southeast Alaska, 1933-1999. Fishery Bulletin 105:234-248

von der Ohe CG, Servheen C (2002) Measuring stress in mammals using fecal glucocorticoids: opportunities and challenges. Wildlife Society Bulletin 30:1215-1225

Waite J, Burkanov V, Andrews R (2012) Prey competition between sympatric Steller sea lions (*Eumetopias jubatus*) and northern fur seals (*Callorhinus ursinus*) on Lovushki Island, Russia. Canadian Journal of Zoology 90:110-127

Wasser SK, Hunt KE, Brown JL, Cooper K, Crockett CM, Bechert U, Millspaugh JJ, Larson S, Monfort SL (2000) A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. General and Comparative Endocrinology 120:260-275

Wilcoxon F (1945) Individual comparisons by ranking methods. Biometrics Bulletin 1:80-83

Wright BE, Riemer SD, Brown RF, Ougzin AM, Bucklin KA (2007) Assessment of harbor seal predation on adult salmonids in a Pacific Northwest estuary. Ecological Applications 17:338-351

GENERAL CONCLUSIONS

Estimating diets of free-ranging predators presents many unique challenges. In this thesis, I have tied together many factors that may impact our understanding of a marine predator's diet, from the predator's foraging strategy, the variability in prey species availability, to the nutritional value of the diet of harbor seals from different sexes and age classes. I also applied new methods to diet estimates of a free-ranging harbor seal (*Phoca vitulina*) population by utilizing software that allows evaluation of the nutritional value of the diet and by identifying sex or age class of the seals sampled.

In the first chapter, yearly average diet composition profiles were constructed for Tugidak Island harbor seals using scat analysis. I estimated diets using two calculation methods, split-sample frequency of occurrence (ssFO) and biomass reconstruction (BR) to demonstrate how interpretation of dominant prey species can differ between estimation methods. Despite the relative biases associated with each method, and even though BR calculations are more time consuming to carry out, the BR methods of estimating consumed diet was likely to provide a better representation of overall diet than ssFO methods based on a review of published literature. Within this study, however, the nutritional value of diets estimated by ssFO and by BR produced similar results. Therefore, because ssFO is easier and less time consuming to calculate than BR, ssFO was an adequate method.

We found that the relative importance of two prey species (gunnel and *Gymnocanthus* spp.) in harbor seal diets was significantly correlated with sea surface temperature (SST) anomalies calculated in the Gulf of Alaska during the collection time period (2001-2009). Harbor seal use of certain prey species such as gunnel and *Gymnocanthus* spp. were negatively correlated with SST and, therefore, in a warming climate, these prey species might become even more

important prey items for harbor seals. However, in this study, neither of these prey species were observed to have a high relative importance to overall diet (relative importance <10%). Future studies should examine the potential effect that other climatic variables, such as sea surface height and increasing freshwater run-off, might have on changes to diet composition. Further research into this field of climatic variables on the diets of apex predators is growing and should benefit ecosystem studies establishing linkages between physical factors and biological consequences.

Despite the fluctuating contribution of multiple prey species across the time series, the nutritional parameters of the average summer diet did not significantly correlate with SST change over time. Therefore, we concluded that even though the prey composition of the diet could be very different from one year to another, harbor seals appeared to be able to maintain a relatively stable nutritional intake during the summer months from 2001-2009. The MIXIT program used to estimate the nutritional value of the diets enables researchers to synthesize prey nutritional data from estimated diets and approximate their nutritional value. This program adds a new level to diet studies by incorporating the nutritional quality of an estimated diet so we are no longer just relying on biomass consumed, but on the nutritional value of prey items consumed as determined by proximate composition. This could be a valuable tool in the future as the ecosystem changes so scientists can evaluate how alterations to the prey base might impact apex predators relying on certain species.

There are several challenges, however, with the current use of the MIXIT program. For the MIXIT program to accurately estimate nutritional quality of diet, an extensive database of prey proximate composition must be used. Some prey species may exhibit large fluctuations in lipid and gross energy density depending on the location and season the samples were collected. However, for most prey species, these data were not available. Therefore, additional research

should focus on seasonal variability of proximate composition of prey as well as changes associated with prey sex and age to use MIXIT to its fullest potential.

Chapter Two explored the use of fecal corticosteroid metabolites to identify seal sex or age classes from scats collected for diet analysis. Fecal corticosterone metabolite profiles of known adult male and known adult female or juvenile male harbor seals split into two distinct groups, which could be matched to profiles identified from unknown sex and age samples. Therefore, we presumed that the profiles that were observed in unknown samples likely represented scats from adult males and from adult females or juveniles. When the diets of these two groups were compared, the diet diversity (as determined by the Shannon diversity index) of the presumed adult males was found to be significantly lower than the diet diversity of the presumed adult females/juveniles. However, the overall nutritional value of the diets of the two groups, as determined by proximate composition, was not significantly different. This indicated that even though these groups were differentially dependent on different prey species, the nutritional value of these diets remained similar.

Future studies should examine known-sex scats from free-ranging populations to account for any variability in steroid metabolites between captive and free-ranging populations or populations that cross large geographic regions, such as harbor seals. This methodology is especially valuable because it enables us to add another dimension to dietary scat analysis by gaining more information about the seal depositing the scat while avoiding more invasive procedures. Furthermore, because scat collections are minimally invasive, it may be the best method to study sex- or age-specific diet differences of at-risk populations, where intrusive sample collections are discouraged.

Overall, I found that a wide variety of factors have the potential to impact harbor seal diet and the available prey base. However, as generalist predators, harbor seals appear to be capable of

maintaining their nutritional intake (based on proximate composition) even in the face of a changing prey base. This suggests that harbor seals may be in a better position to cope with a changing ecosystem than a predator that specializes on one particular prey item. Dietary data are limited for harbor seals before their decline in the 1970s and 1980s, so it is difficult to assess if a change in their diets contributed to the decline. This study provided baseline data on a harbor seal population that is currently increasing. Therefore, if the Tugidak Island harbor seal population experiences another decline, we may be better able to compare the diets of the harbor seals in increasing and decreasing periods to evaluate the potential effect of changing diet to the decline.

APPENDICES

Appendix 1: Parameters used for length-weight regression equations. Length-weight regression equations used to estimate consumed biomass from estimated prey length. The equation used was $W = aL^b$ and species-specific constants for "a" and "b" are shown in Table A0.1 below. The length column represents the unit of measure that should be used when entering length for the equation. All weights estimated were in grams. For prey species where multiple length-weight equations could be located, entries were selected based on larger sample sizes, and when two entries were located with comparable sample sizes, the entry was chosen based on sample collection location.

Species	A	B	Length	*N*	Location	Citation[12]
Armorhead Sculpin	0.010000000	3.1960	cm	29	NE Pacific Ocean	Harvey 2000
Arrowtooth Flounder	0.009300000	2.9990	cm	101	NE Pacific Ocean	Harvey 2000
Capelin	0.000000020	4.2304	mm	191	Prince William Sound	Brown 2002
Dover Sole	0.009400000	3.0920	cm	101	NE Pacific Ocean	Harvey 2000
Eulachon	0.007700000	3.0750	cm	129	NE Pacific Ocean	Harvey 2000
Greenling	0.000002000	3.3399	mm	4	NE Pacific Ocean	Van Pelt et al. 1997; From Litzow et al. 2002
Gunnel	0.000000800	3.2825	mm	42	Gulf of Alaska	Anthony et al. 2000; From Litzow et al. 2002
Pacific Cod	0.000002772	3.2180	mm	764	Aleutian Islands	Rooper 2008
Pacific Halibut	0.007460000	3.0930	cm	1830	Eastern Bering Sea	Fadeev 2005
Pacific Sandfish	0.017000000	2.9530	cm	19	NE Pacific Ocean	Harvey 2000
Poacher	0.004300000	3.1258	cm	229	Western Bering Sea	Glubokov & Orlov 2008
Rex Sole	0.023800000	2.6920	cm	67	NE Pacific Ocean	Harvey 2000
Rock Sole	0.011200000	2.9970	cm	83	NE Pacific Ocean	Harvey 2000
Ronquil	0.000003000	3.1770	mm	8	Gulf of Alaska	Anthony et al. 2000; From Litzow et al. 2002

[12] Full reference can be found in list of literature cited following appendices.

Species	A	B	Length	*N*
Salmon	0.000006000	3.0781	mm	51
Sand Lance	0.000002000	3.1224	mm	1155
Searcher	0.003800000	3.2560	cm	44
Pollock	0.004300000	3.2550	cm	46

Location	Citation[12]
Gulf of Alaska	Anthony et al. 2000; From Litzow et al. 2002
Gulf of Alaska	Robards et al. 1999a; From Litzow et al. 2002
NE Pacific Ocean	Harvey 2000
NE Pacific Ocean	Harvey 2000

Appendix 2: Proximate composition values used for nutritional analysis of estimated diets. All nutritional parameters were entered into the MIXIT-WIN program as dry-matter (DM) values and the MIXIT program presents results as wet-matter (WM) values. Conversions between WM and DM data were calculated using Equation 3 in Appendix 3. The proximate composition data listed in Table A2 are presented as wet-matter values. Appropriate equations were used to calculate necessary data fields from provided data and these calculations are noted where applied and the equations are located in Appendix 3.

Species	Protein (%)	Lipid (%)	Ash (%)	Water (%)	GE (kJ/g)	Citation[13]	Comments
Armorhead Sculpin	13.10	4.20	1.99	73.90	4.770	Anthony et al. 2000	Protein calculated using Equation 1; Ash calculated using Equation 2 (Appendix 3)
Arrowtooth Flounder	17.70	2.30	1.11	79.50	5.104	Stansby 1976	GE calculated using Equation 1 (Appendix 3)
Bering Poacher	11.12	0.86	4.78	79.50	2.973	Ball et al. 2007	Protein calculated using Equation 1 (Appendix 3)
Capelin	14.26	4.76	2.56	78.90	5.267	Payne et al. 1999	
Chinook Salmon	19.50	11.50	1.28	73.10	9.186	Stansby 1976	GE calculated using Equation 1 (Appendix 3)
Chum Salmon	21.30	3.86	1.18	74.10	6.573	Stansby 1976	GE calculated using Equation 1 (Appendix 3)
Coho Salmon	21.70	5.31	1.21	72.60	7.247	Stansby 1976	GE calculated using Equation 1 (Appendix 3)
Dover Sole	16.58	7.79	3.01	71.32	7.118	Bando 2002	
Eulachon	11.95	18.82	1.55	68.09	10.312	Payne et al. 1999	
Giant Pacific Octopus	10.77	0.82	1.96	85.61	2.931	Bando 2002	
Greenling	15.13	1.78	3.41	79.33	3.431	Van Pelt et al. 1997	
Gunnel	14.78	3.00	1.51	73.90	4.690	Anthony et al. 2000	Protein calculated using Equation 1; Ash calculated using Equation 2 (Appendix 3)
Pacific Cod	16.38	2.05	2.19	79.60	4.050	Logerwell & Schaufler 2005	

[13] Full reference can be found in list of literature cited following appendices.

Species	Protein (%)	Lipid (%)	Ash (%)	Water (%)	GE (kJ/g)
Pacific Halibut	20.70	0.79	1.35	78.30	5.213
Pacific Sandfish	15.03	3.82	3.04	78.17	5.074
Pink Salmon	19.00	4.76	1.20	75.60	6.389
Red Irish Lord	13.82	1.46	3.36	75.20	3.849
Rex Sole	16.70	0.71	1.10	82.30	4.233
Ribbed Sculpin	12.64	4.56	2.65	72.70	4.799
Rock Sole	19.00	6.00	4.00	71.00	7.000
Ronquil	13.16	2.50	2.26	76.60	4.110
Salmon (Unknown)	16.47	3.63	2.25	75.66	4.639
Sand Lance (Aug.	17.84	5.68	2.42	73.10	5.408
Sand Lance (July)	16.11	7.10	2.25	73.05	5.659
Sand Lance (June)	16.43	6.60	2.45	73.35	5.515
Sand Lance (September)	17.46	4.77	2.53	74.35	4.986
Searcher	13.13	2.86	2.36	75.80	4.238
Sockeye Salmon	21.30	8.55	1.18	70.00	8.441
Pollock	14.99	5.30	2.55	75.90	5.357

Citation[13]	Comments
Stansby 1976	GE calculated using Equation 1 (Appendix 3)
Payne et al. 1999	
Stansby 1976	GE calculated using Equation 1 (Appendix 3)
Anthony et al. 2000	Protein calculated using Equation 1; Ash calculated using Equation 2 (Appendix 3)
Stansby 1976	GE calculated using Equation 1 (Appendix 3)
Anthony et al. 2000	Protein calculated using Equation 1; Ash calculated using Equation 2 (Appendix 3)
Worthy & Miculka 1997	
Anthony et al. 2000	Protein calculated using Equation 1; Ash calculated using Equation 2 (Appendix 3)
Logerwell & Schaufler 2005	
Robards et al. 1999b	
Robards et al. 1999b	
Robards et al. 1999b	
Robards et al. 1999b	
Anthony et al. 2000	Protein calculated using Equation 1; Ash calculated using Equation 2 (Appendix 3)
Stansby 1976	GE calculated using Equation 1 (Appendix 3)
Kitts et al. 2004	

Appendix 3: Equations used to calculate unreported proximate composition values. Wet-matter protein content and gross energy (GE) was calculated using Equation 1 which used commonly accepted coefficients for protein and lipid energetic value Payne et al. 1999. Energetic contribution of carbohydrates is considered negligible in fish and was not included in our calculations. GE was then converted to kJ g^{-1} (1kcal g^{-1}=4.2kJ g^{-1}). Wet-matter ash content was calculated using Equation 2. Dry matter (DM) and wet matter (WM) values were calculated using Equation 3.

Equation 1:

$$GE \text{ (kcal g}^{-1}) = (\ \%\ Protein\ Content \times 5.65) + (\ \%\ Lipid\ Content + 9.50)$$

Equation 2:

$$Ash\ (\%\ DM) = \%\ DM\ - Protein\ (\%\ DM) - Lipid\ (\%\ DM)$$

Equation 3:

$$P_D = \frac{P_W}{Dry\ Matter\ (\%) \div 100}$$

Where P_D is the nutritional parameter (i.e., percent protein or lipid) on a DM basis and P_W is the same nutritional parameter on a WM basis.

LITERATURE CITED

Anthony J, Roby D, Turco K (2000) Lipid content and energy density of forage fishes from the northern Gulf of Alaska. Journal of Experimental Marine Biology and Ecology 248:53-78

Ball JR, Esler D, Schmutz JA (2007) Proximate composition, energetic value, and relative abundance of prey fish from the inshore eastern Bering Sea: implications for piscivorous predators. Polar Biology 30:699-708

Bando MKH (2002) Comparing the nutritional quality of Steller sea lion (*Eumetopias jubatus*) diets. Master of Science, University of Alaska Fairbanks, Fairbanks, AK, p54

Brown ED (2002) Life history, distribution, and size structure of Pacific capelin in Prince William Sound and the northern Gulf of Alaska. ICES Journal of Marine Science: Journal du Conseil 59:983-996

Fadeev N (2005) Guide to biology and fisheries of fishes of the North Pacific Ocean. TINRO-Center, Vladivostok, Russia

Glubokov A, Orlov A (2008) Data on distribution and biology of poachers agonidae from the northwestern part of the Bering Sea. Journal of Ichthyology 48:426-442

Harvey JT (2000) Relationship between fish size and otolith length for 63 species of fishes from the eastern North Pacific Ocean. National Marine Fisheries Service, NOAA Technical Report

Kitts D, Huynh M, Hu C, Trites A (2004) Season variation in nutrient composition of Alaskan walleye pollock. Canadian Journal of Zoology 82:1408-1415

Litzow MA, Piatt JF, Prichard AK, Roby DD (2002) Response of pigeon guillemots to variable abundance of high-lipid and low-lipid prey. Oecologia 132:286-295

Logerwell EA, Schaufler LE (2005) New data on proximate composition and energy density of Steller sea lion (*Eumetopias jubatus*) prey fills seasonal and geographic gaps in existing information. Aquatic Mammals 31:62-82

Payne SA, Johnson BA, Otto RS (1999) Proximate composition of some north-eastern Pacific forage fish species. Fisheries Oceanography 8:159-177

Robards M, Piatt J, Rose G (1999a) Maturation, fecundity, and intertidal spawning of Pacific sand lance in the northern Gulf of Alaska. Journal of Fish Biology 54:1050-1068

Robards MD, Anthony JA, Rose GA, Piatt JF (1999b) Changes in proximate composition and somatic energy content for Pacific sand lance (*Ammodytes hexapterus*) from Kachemak Bay, Alaska relative to maturity and season. Journal of Experimental Marine Biology and Ecology 242:245-258

Rooper CN (2008) Data Report: 2006 Aleutian Islands Bottom Trawl Survey. US Dept. of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Alaska Fisheries Science Center p1-253

Stansby ME (1976) Chemical characteristics of fish caught in the northeast Pacific Ocean. Marine Fisheries Review 38:1-11

Van Pelt T, Piatt J, Lance B, Roby D (1997) Proximate composition and energy density of some North Pacific forage fishes. Comparative Biochemistry and Physiology-Part A: Physiology 118:1393-1398

Worthy GAJ, Miculka TA (1997) Proximate composition and fatty acid signatures of selected forage fish species in Prince William Sound, Alaska. Exxon Valdez Oil Spill Trustee Council, (Restoration Project 95121). National Oceanic and Atmospheric Administration, Seattle, WA